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Losses of Potatoes in Cold Storage *Vis-a-Vis* Types, Mechanism and Influential Factors

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Losses of potatoes in cold storage occur in all the potato-producing countries and are caused, in part, by excessive high temperatures during storage, which increase rotting, decay and loss of edible quality and nutritive values. In some cases, freezing or chilling injury, also called cold injury, caused by low temperatures may be involved. Other serious losses are caused by mechanical injury from careless or rough handling and by shrinkage or wilting because of moisture loss. Diseases and pests, which develop during favourable conditions in cold storage, may also cause losses in the form of rottage, decay, poor germination and deformation. The extent of losses of potatoes in cold storage depends on a number of factors and the most important ones are temperature, humidity, ventilation and the condition of potatoes stored. Therefore, the matter of reducing the storage losses directly leads to the problem of providing more efficient and appropriate storage arrangements for potatoes. An attempt has been made in this review, to critically appraise the types of losses of potatoes in cold storage, their causes, preventive measures and important factors affecting the extent of these losses.

Keywords : Potato, Cold storage, Storage losses, Stacking, Temperature, Relative humidity.

In order to reduce the deterioration of semi-perishable potatoes immediately after harvest, they are cooked from their ambient conditions to their optimal storage temperature around 4-8°C. This is generally achieved in a cold store for long-term storage. This is the method of preservation of semi-perishable potatoes in their fresh and wholesome state for extended periods by providing and controlling proper temperature and humidity conditions within the storage compartment. The factors responsible for physical and chemical changes causing spoilage of potatoes may be rendered dormant or substantially retarded in its activity by exposing them to low temperature conditions. The cold storage is serving mankind by preventing the spoilage of potatoes and making them available during off-season and in places, where they are not harvested at all. This also serves dual purposes : the growers of the potatoes need not require to sell out their produce in a hurry at a throwaway price and the nation need not strive from shortage of the produce due to spoilage during off-season.

Commercially, cold storage for potatoes was started in India in the thirties of the last century. Since then, the number of cold stores and their installed capacity is increasing continuously. In India, at present, there are about 3443 cold store units (Ezekiel et al. 1999). Out of this, 2975 are in the private sector and 303 in the cooperative sector. The present cold storage facility in India is around 31 million cubic meters, capable of storing 10.35 million tonnes (Das 2000). Out of this, about 85-90% capacity being exclusively used for potatoes only (Maheshwari 1979; Shekhawat 1994). This is sufficient only to accommodate 46% of the total potato production against the ideal requirement of 50-60% (Shekhawat and Dahiya 1997).

Potato loss in cold storage is a problem confronting all the potato producing countries including India. Data on potato losses in some of the developing countries are given in Table 1. Total potato losses in the world may range from 5-40% of which on an average, 8% are only in the cold storage (Garg et al. 1979). In India, the storage losses in potato cold storages account for 3-10% of the stored product in the form of rotting, cold injury, weight loss, sprouting, nutritive value degradation, etc. However, the potato losses may increase up to 38-45% due to defective cold storage infrastructure and management (Bhatnagar and Gupta 1979). These losses attributed to the fluctuating temperatures in the cold store, overloading of the potatoes, adoption of cheap but outdated technology of the cold storage and its poor functioning as well as improper management of the cold store. The problem of losses is very severe in an environment of high temperature and high humidity. The losses depend upon the stacking arrangement, loading density, free area available for heat transfer, air velocity in cold storage, storage temperature, humidity, ventilation, condition of the product stored, geometry of the bag, packaging, etc.

In a country like India, cold storage is an economic necessity, where in the absence of adequate storage facilities, there is wastage of this economically important crop in the order of around 20%. To sustain the increasing potato production, it has been estimated that the additional requirement of cold storage capacity in the next five years will be around 1.2 million

TABLE 1. POTATO LOSSES DURING STORAGE IN SOME OF THE DEVELOPING COUNTRIES

Name of the country	Losses, %
Bolivia	25
Brazil	5 - 30
Chile	30
Ghana	10 - 20
Iran	5 -100
Nigeria	15 - 50
Peru	20 - 50
Rwanda	5 - 40

Garg et al (1979)

* *Corresponding Author*

tonnes (CPRI 1999). Since there is inadequate inflow of capital in potato cold storage industry, one of the ways to attract more investments in the cold storage sector is to strive constantly towards the reduction of storage losses. There is a considerable scope to reduce these storage losses. The foregoing discussion necessitates a critical review of the types of losses in the potato cold storage, their mechanism, ways to reduce it and the factors governing the extent of these losses. This has been attempted in the following sections with special reference to India.

Types of storage losses and their mechanism

The proper storage environment for potatoes may be defined as the environment, which will promote the most rapid healing of bruises and cuts, reduce rot penetration to a minimum, allow the least mass and other storage losses to occur and reduce to a minimum the deleterious quality changes that might occur during cold storage (ASHRAE 1990). The losses observed during potato cold storage can broadly be divided into two groups, viz., quantitative losses and qualitative losses. The difference between these two groups of losses has only theoretical significance because they occur simultaneously and mutually affect one another. Storage losses are mainly caused by the following reasons.

Losses due to high temperature

Rotting : A fairly important storage loss due to high temperature is rotting of the potatoes. The centre-most product in a stack is more frequently subjected to rotting, where high temperature is developed due to long cool-down time (time required to bring the potatoes at desired storage temperature) and high respiration heat of potatoes. To store the potatoes for a long time, they are generally packed in gunny bags and then these bags are arranged in stacks within the cold store. The process of cooling to achieve the desired storage temperature of the potatoes requires long time, generally in the order of 15-30 days and sometimes it may be up to 45 days (Anon 1997). This is attributed to the large stack dimensions and low thermal conductivity of the stack. The fans located on the top of the cold store near the evaporator are inadequate to circulate the air throughout the cold store. Therefore, the heat transfer from potato bags to the storage air takes place by the natural convection process only, resulting to a very low heat transfer coefficient. Also, at the beginning of storage, the rate of respiration is high at higher temperatures. Thus, the centre-most potatoes initially take long time in cooling due to poor overall heat transfer coefficient. Moreover, due to high respiration rate, temperature further rises, leading to spoilage. If this cool-down time is more than the time required for the initiation of deterioration, the rotting phenomenon starts.

High overall heat transfer coefficient or low cool-down time can be achieved by lowering the storage air temperature (increasing the temperature gradient), increasing the storage air velocity and limiting the size of the stack as small as economically feasible.

Blackheart : Another form of storage loss due to high temperature is blackheart. The high temperature, which causes blackheart may develop due to the above mentioned reasons. Blackheart occurs as a result of sub-oxidation. Potatoes begin to respire strongly at a temperature of 30°C or higher, the tubers require a great deal of oxygen and develop large amount of carbon dioxide. At a certain temperature, respiration may become so intensive that the cells deep inside the tuber can no longer obtain sufficient oxygen and the carbon dioxide formed can not be released. The cells then die, giving rise to blackheart. Under adequate supply of air, this disorder does not occur, unless temperatures are 32°C or higher. An air removal of $10 m^3$ / tonne / 24 h is sufficient to avoid the occurrence of blackheart at cold store temperature. According to Ramsey et al (1949), this defect may be prevented by supplying adequate air by ventilation and keeping the temperature below 27°C. The internal symptoms of blackheart are dark grey to purplish or black discoloration near the centre. Varieties like 'Kufri Kuber', 'Craigs Defiance', 'Darjeeling Red Round', 'Hybrid ON 45' and 'Hybrid 555' are particularly susceptible to blackheart (Pushkarnath 1976).

Losses due to extremely low temperature

Cold injury : The potatoes near the surface of bag achieve the temperature close to the storage air temperature, relatively in short time and then, these parts of the potatoes remain at this temperature for rest of the storage period (Chourasia 1997). If temperature of the potatoes achieved is lower than the threshold temperature, it causes cold injury (an injury caused by exposure to low but non-freezing temperature, often below 3°C). The chilled potatoes become weakened because they are unable to carry on normal metabolic processes. Often, potatoes that are chilled look sound, when removed from low temperatures. However, symptoms of chilling become evident in a few days at warmer temperatures (Morris et al. 1938).

To investigate the low temperature effects, Cunningham et al (1976) chilled tubers at –3.8°C, –2.5°C, and –1.1°C for various time periods and then held at 7.2°C for 1 to 4 weeks before testing. Susceptibility to injury was quite similar between the tubers of two seasons and among tubers of the same lot exposed to the same treatment. Greatest amount of low temperature breakdown was occurred in tubers chilled at –2.5°C for 10 h. Flesh colour, reducing sugar contents, mealiness, and amount of sloughing in tubers that survived chilling were not markedly different from those in control samples. Certain trends, however, were observed, indicating that the quality of the chilled tubers was beginning to change. Chilling increased yellowness of flesh colour in most treatments and darkness in several treatments. Some varieties like 'Kufri Kuber' are susceptible to cold injury and may show tuber injury under prolonged storage conditions at temperatures even somewhat above freezing point. Some varieties, like 'Phulwa', do not show tuber injury even if cold storage temperatures drop by a degree or so below the freezing point (Pushkarnath 1976).

Mahogany browning : In mahogany browning, internal discoloration may occur in irregular patches anywhere in the tubers. Affected tubers have normal firmness and consistency but are unfit for processing. This defect develops when tubers are stored near 0°C for 20 weeks or longer. Mahogany browning is found in certain varieties like 'Katahdin', when stored over several weeks at temperatures near about freezing point.

Mahogany browning may be avoided by storing tubers at 3.3°C or above (Hilborn and Bonde 1942).

It is clear that both the time and temperature are involved in chilling injury as well as mahogany browning. Damage may occur in a short time, if temperatures are considerably below the danger line, but some potato varieties may withstand a few degrees in the danger zone for a longer time. Also, the effects of chilling are cumulative. Low temperatures in the field before harvesting (in the plains of India during the months of December-January) and in transit, add to the total effects of chilling that might occur during storage. Good insulation, air circulation, and artificial heating, if necessary, during transit and storage may prevent this type of injury (Ramsey et al. 1949).

Losses due to diseases

Invasion of microorganisms into the tubers may cause considerable losses due to diseases. The diseases, which develop during storage are late blight, early blight, dry rot, pink rot, gangrene, skin spot, black dot, silver scurf, charcoal rot, soft rot, etc (Nagaich et al. 1979). High temperature and high relative humidity are important factors responsible for disease development during cold storage. Normally, infection takes place in the field and in most of the occasions injuries of the tubers become the entry points to the microorganisms. Infection by some microorganisms may, under favourable storage conditions, cause rapid rotting of the potatoes. Although the losses may be negligible or low (below 5%) in cold stores, even entire material may be lost under conditions of high temperature developed accidentally due to defective operation and management of refrigeration system (Singh and Verma 1979). When adjacent healthy tubers are affected, there will be occurrence of so-called clusters. Once these clusters occur, the rotting can not be prevented any longer. The rotten tubers stick to each other, due to which ventilation of the tubers on top is no longer possible. As apparently, the affected tubers also produce extra heat, temperature rises very rapidly and within a few days, the entire potato stack may have become rotten (Sparenberg 1979). To counteract the spread of diseases in the cold store, care must first be taken at the beginning of storage period. Also, the potatoes must be maintained at desired low temperatures and kept dry throughout the storage.

Weight loss of the potato

Potatoes shrink or lose weight during cold storage. Potato tuber, being a living organism, undergoes metabolic changes as a result of vital functions. Admittedly, such changes are considerably weaker during the dormancy period, nevertheless they can affect the level of weight loss considerably. The most important means for losses are evaporation, respiration and sprouting. The agents, which influence the extent of weight loss are - (i) stage of the maturity at the time of harvest (ii) quality of the potato skin as well as injury during harvesting and storing (iii) storage temperature and humidity during a short time after harvesting (iv) temperature, humidity, and amount of ventilation during storage and (v) length of storage as well as subsequent sprouting.

Smith (1933) found that potatoes harvested at immature stage, lost 9.89% in weight during 7 months storage, whereas potatoes harvested, when mature, lost 6.97% during the same period. Potatoes handled carefully during harvesting and storage lost 6.78% on 7 months of storage; while those harvested in the normal manner lost 10.08%. Potatoes cured for 8-12 days immediately after harvest under warm, moist storage conditions lost 7.54% of their weight and those not cured had a weight loss of 9.33% on 7 months of storage. At the end of 1 month storage, mature tubers lost 4.66% in weight; 6.41% after 3 months; 7.74% after 6 months; and 8.91% at the end of 7 months.

The permeability of water through periderm and thus also the degree of suberization, plays a very important part in limiting moisture loss during cold storage (Es van and Hartmans 1989 a). Water loss in badly suberized and damaged potatoes is very high. Water evaporates through the skin, wounds and sprouts in the ratio of 1:300:100. Therefore, it is important to stimulate suberization and wound healing in the tubers right at the beginning of the storage season by keeping the potatoes at a temperature of 15 to 20°C and a relative humidity of 95% or more. Minimum possible ventilation should be done during this 7 to 14 days curing period. After this, the potatoes must be cooled to the required storage temperature as quickly as possible.

High relative humidity, as high as 100%, for 2 weeks after harvest kept the weight losses down in subsequent storage. Relative humidity is more important than temperature, provided temperatures do not go below 7.2°C (Schippers 1971a, b). The water loss per square centimetre of skin area per hour per millimetre of mercury vapour pressure deficit (VPD), shortly after harvest, was five to seven times as high as later part of the storage period (Schippers 1971c). Burton (1966) calculated weight losses of 0.01-0.015 mg/cm^2/mm of mercury VPD for mature tubers that had been stored at 10°C for about 1 month after harvest. Van den Berg and Lentz (1973) reported that optimum relative humidity for potato storage at 4-8°C was 98-100%. Butchbaker et al (1973) reported that age of tubers and temperatures were most highly correlated with weight loss. Tuber weight and relative humidity had the next strongest correlation with weight loss and air velocity had the lowest correlation. Samotus et al (1973) found that after 22 weeks of storage at 1°C, the losses were about twice those of reconditioning potatoes for 2 weeks at 20°C.

The loss in weight is directly proportional to the temperature and the period of storage. Karmakar and Joshi (1950) determined losses over a period of time at 1.7° and 4.4°C as shown in

TABLE 2. LOSS IN WEIGHT OF POTATOES AT DIFFERENT PERIODS OF COLD STORAGE

Period of storage, weeks	Loss in weight, %	
	1.7°C	4.4°C
1	2.66	2.99
3	5.60	5.71
5	7.20	7.13
7	8.85	9.06
9	10.69	12.11

Karmakar and Joshi (1950)

Table 2. The degree of sprouting is also important in relation to the weight losses. Burton (1955) calculated that the epidermis of the spouts is about 100 times as permeable to water as the surface of the tuber. Hence, sprout growth corresponding to a 1% increase in the tuber area causes a doubling of the moisture loss. For every 1% by weight of sprouts, the free moisture loss to the air increases by 0.075% of the initial tuber weight per week per millibar of vapour pressure deficit (Burton and Hannan 1957). The sprout, thus, has a relatively substantial effect on moisture loss.

Evaporation : In evaporation, water is lost from the potatoes, so that the weight of the product remaining for sale is smaller. This water loss is, thus, principally an economic loss and the original food value of the potatoes remains virtually unaffected. However, large water loss may adversely affect the quality of the potatoes. Tubers, which have lost a lot of water, are more susceptible to mechanical damage, such as bruising and blue discoloration. High water loss also leads to greater peeling losses, reduction of culinary quality, etc. In economic terms, it is, therefore, very important to limit water loss.

The moisture loss in the cold storage is dependent on the extent to which water inside the tuber is free for transport to the outside (storage atmosphere) and the extent to which the outside can absorb this water as well as on the coefficient of transpiration. To lower the storage air temperature, evaporator temperature is lowered. As the evaporator temperature decreases, the partial pressure of the water vapour near the evaporator surface decreases (RH remaining constant), effecting more migration of moisture from the product to the evaporator. This causes dehydration (weight loss due to reduction in moisture content) of the potatoes. The migrated moisture freezes on the evaporator surface, which decreases the heat transfer rate on the evaporator surface and increases the defrosting time. The moisture loss may be minimised by using moisture preventive plastic packaging material. But the plastic film, if sealed or tightly tied, may restrict carbon dioxide, oxygen and water vapour, leading to harmful concentrations of these respiratory gases. Plastic film also restricts heat transfer, which retards the rate of cooling (Hardenburg 1971). Waxing is also not recommended for potatoes either before or after storage (Hardengurg et al. 1959).

Potato tubers immediately after harvest lose water three times faster than they do after a month of storage. Such a great difference is due to the fact that potato skin, after harvest, is thin and loosely bound with flesh. After a preliminary period of storage, the skin is covered with a cork layer, which retards water evaporation. During this period, damage and injuries caused during harvesting and transport are being repaired. The next period of rapid water evaporation from the tubers starts together with sprouting. Water evaporation from the tubers during cold storage accounts for the quantitative losses and is detrimental to potato consistency. Hence, evaporation should be minimized. This can be achieved by providing the atmosphere in the cold store with a high humidity (80-90%). The poratoes stored under such conditions can neither become too dry nor absorb too much water. Relative humidity below 80% leads to drying of the potato tubers. Such tubers are usually shrivelled, lose compactness and become soft. Dampness at high temperature, on the other hand, favours the growth of microorganisms, causing mould and rotting of the potatoes.

Respiration : Respiration is a central process that occurs in all living cells including the potato. In the respiration process, the substrates used are oxygen and starch. Carbon dioxide, water and energy are the end products. Because these mostly are released into the surrounding atmosphere, the overall effect is a loss in weight of the stored potato. A simplified representation of the process is -

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + 2667 \text{ kJ energy}$$

The amount of released carbon dioxide is a measure of respiration intensity. According to Smith (1933), the loss in weight of potatoes in cold storage due to respiration was very small (about 1/10) in comparison to the loss in weight caused by evaporation of water. In common storage of 7 months period, shrinkage, as a result of respiration was only 0.51%, whereas shrinkage due to water loss was 5.71%. In the temperature range of 5-10°C, these losses seldom exceed 1.5% of the fresh weight of the potatoes in a storage period of 6 to 8 months, which is equivalent to a loss of 6 to 7% of the total dry matter of the potatoes. However, this dry matter loss does not affect the total weight loss of the potatoes appreciably because a part of the dry matter used in respiration is converted into water, which remains in the potato (Rastovski 1989 a). These losses were subsequently greater at higher storage temperatures.

Sprouting : During the development of the tubers on the plant, the buds in the eyes on the tuber successively become dormant, starting at the stem end. At the end of the dormant period, the eyes again sprout. If the tubers are placed under appropriate temperature conditions, one or more sprouts form on each tuber. The physiological condition of the tuber, diseases and storage conditions greatly affect sprout growth during cold storage. The length of the dormancy period and sprouting behaviour depend on the cultivar and on the previous history of the tubers. Storage conditions such as temperature, humidity of the storage atmosphere, light, oxygen concentration, carbon dioxide concentration and the possible presence of the volatile substances from the potato also affect the length of the dormancy period and sprouting.

Temperature strongly influences the duration of the dormancy period and subsequent sprouting. There is direct correlation between the storage temperatures and the time taken for sprouting. The expected time taken for sprouting by freshly harvested potatoes held in cold storage at different temperatures is given in Table 3. Reducing the storage temperature of potatoes from 10 to 3°C increased the dormancy period by 150%. It is also important for the temperature not to vary too much, as this generally reduces the dormancy period. The moistening reduces the period of dormancy. But this could

TABLE 3. AVERAGE TIME TAKEN FOR SPROUT INITIATION AT VARYING STORAGE TEMPERATURES

Storage temperature, °C	5	10	15	20
Time taken for sprout initiation, weeks	30	15	8	5

Pushkarnath (1976)

just as well be caused by a reduction in the oxygen pressure due to water film (Burton and Wigginton 1970).

The composition of storage atmosphere also affects the sprouting process to a great extent. The oxygen content affects not only termination of dormancy but also the rate of sprout growth. The oxygen concentration required for optimum sprout growth depends on the physiological age of the potatoes. The low oxygen concentration, which has a stimulating effect at the beginning of storage, clearly inhibits sprout growth later in the season. To induce sprouting, an oxygen content of 2% is sufficient at the beginning of the storage. However, the sprouts then either fail to grow, or grow only slightly. This can be attributed to the inability to mobilize the reserve carbohydrates for sprout growth. A storage atmosphere with a carbon dioxide content of 2.2 to 9.1% clearly stimulated sprout growth in potatoes irrespective of the stage of dormancy. Volatile substances, released during the storage period, may inhibit sprouting even at normal oxygen and carbon dioxide concentrations (Burton 1965; Burton and Meigh 1971).

Losses due to defects

Mechanical damage : This is one of the most common defects. Increasing mechanization in harvesting, transport to and from the cold store and all operations associated with grading and marketing have resulted in a considerable increase in damage. The following clearly demonstrable causes may be mentioned : incorrectly equipped or adjusted lifting machines, excessive dropping heights, etc. Dropping heights must be limited to 30 - 40 cm. Mechanical damage increases with increasing the dry matter content (Meijers 1989).

The damage often becomes visible only after several days or weeks. A typical form is hard, dry grey-white mass of starch from the damaged cells, evident on cutting. Tuber damage gives rise to an increase in the respiration rate and in evaporation losses. In addition, the quality of the lot is reduced and skin and flesh losses are greatly increased. Again, these damaged areas frequently constitute a point of entry for microorganisms.

Blue discoloration or black spot : Tubers with this defect display local blue-grey discoloration of the potato tissue on cutting. This discoloration is almost always to be found just below the skin around the vascular bundle tissue. However, the blue colour may go much deeper in tubers, which are very susceptible to this condition ('worn out' tubers). The discoloration occurs principally at the stem end. The condition is usually not visible externally. If only, the tuber is very seriously affected, the discoloration can then be observed through the skin below the sunken patch.

The discoloration of the potato tissue is caused by oxidation of certain phenols by the enzyme, phenol oxidase. In this reaction, the blue-black pigment melanin is formed via the red-coloured intermediate product dopachrome. Oxygen is required for this purpose, but can only be supplied after the cells have been damaged. Shortly after the damage occurs, a red discoloration of the tissue occurs first; the blue coloration only becomes visible a few hours or even one or two days later (Meijers 1989). High storage temperatures after the damage accelerate the development of the blue discoloration (Dwelle and Stallknecht 1976). However, at low temperatures the tubers are more susceptible to damage. Therefore, it is recommended that the lots, depending on their susceptibility to black spot, be warmed before handling to a minimum of 12 to 15°C.

The susceptibility of the tubers to blue discoloration increases during cold storage. This may be attributed to the loss of moisture and tissue tension due to evaporation. The tubers at the bottom of the cold store are most susceptible to blue discoloration, because this is the place where high moisture loss occurs (Meijers 1989). If the tuber has not yet sustained any moisture loss, the tissue tension will be optimal. As the tuber increasingly loses moisture, the tissue tension declines and susceptibility to blue discoloration increases (Kunkel and Gardner 1965).

Bruising or pressure spot : One of the consequences of increasing stack heights above 3 m is the occurrence of bruising. In case, tubers have been in contact with each other during storage, the surfaces may be flattened and indented. These indentations are known in practice as bruises or pressure spots because they result from the pressure exerted by the potatoes on each other. The effect of storage height on bruising and weight loss is given in Table 4.

The flesh under a bruise is softer and can be cut rather less easily. Discoloration under the bruise is rarely observed immediately after the store is emptied. The smaller bruises disappear almost entirely after a few days and the deeper indentations partially so. Depending on the severity of the defect, the tissue under a bruise is more or less badly deformed and damaged. In very bad cases, the starch grains are cemented together. When the pressure is removed on emptying the store, the tissue partially spring back, giving rise to small cavities in the tissue in the case of severe bruises. The deformed and sometimes damaged tissue is particularly sensitive to dropping and impact, so that a blue-grey discoloration under the bruises may be observed very soon after grading. It is precisely this characteristic, the high sensitivity to discoloration, which makes bruising such a badly great feared defect. The fact that discoloration can be seen under a bruise very rarely, when the store is emptied, is probably attributable to expulsion of all air, and hence also oxygen, from the tissue by the pressure. When the pressure is relieved and if cavities form, air can return and the discoloration can then occur.

TABLE 4. WEIGHT LOSSES AND BRUISING AT DIFFERENT HEIGHTS IN THE POTATO STACK WITH (A) AND WITHOUT (B) HUMIDIFICATION (STORAGE TEMPERATURE 3 - 5°C)

Height above ground, m	Weight losses, kg/tonne		Bruising, % tubers	
	A	B	A	B
0.25	29	37	24	64
0.50	22	31	23	51
1.00	20	27	19	39
1.50	18	28	12	32
2.00	23	26	7	11
2.50	18	23	2	10
3.00	17	27	0	1

Meijers (1989)

Meijers (1989) has shown that not only pressure but also weight loss can play a very important part. Not only pressure but also weight losses are highest at the bottom of the store (Table 4) and it is, therefore, clear that this combination will often give rise to a very large number of bruised tubers particularly in the bottom meter of the store.

One approach to reduce prevention of this condition would be to reduce the stacking height, but this is not practicable. It is better to start with well-hardened tubers on harvesting. These should be ventilated as little as possible and then, only at high atmospheric humidity, followed by careful grading and transport at tuber temperature, which is not too low. Humidification of the ventilation air has a favourable effect, greatly reducing the proportion of tubers with severe bruises.

Greening : Exposure of tubers to natural or artificial light during post-harvest handling and in storage may cause greening of the skin and cortex. The green tissue is usually accompanied by formation of solanine, an alkaloid that may be poisonous, if consumed in sufficient amounts. Such potatoes have a bitter taste and, when processed, may also result in a less attractive product because of the green colour. To avoid greening in cold storage, lights should not be left on any longer than is necessary.

Losses due to nutritive value degradation

Quality is the most important consideration, when potatoes are stored for table use and processing. The commodity should retain its natural characteristics as regards cooking quality, taste and flavour. After harvest, several physiological changes take place within the tuber. While cold storage helps to prolong the rest period and arrest sprouting, pronounced physiologic changes take place within the tuber and it is hardly possible to avoid these processes. Potato storage, even under the best conditions, affects the chemical composition of the tubers. The qualitative features of potatoes, such as dry matter content, the content of carbohydrate and nitrogen compounds, vitamin content, level of organic acids and susceptibility of the tuber flesh to change in colour, can depend on various factors occurring during potato cold storage.

Specific gravity : The specific gravity of potatoes used for processing is of great importance. High specific gravity potatoes are preferred for potato chips, French fries, and dehydration. On the other hand, potatoes of low specific gravity (below 1.08) are preferred for canning because they slough or fall apart less during processing than potatoes of higher specific gravity (Nutting 1950).

Change in the specific gravity of the tubers depends on the temperature and relative humidity in the storage house as well as storage time (Schippers 1971c). Potatoes stored at a relative humidity of 83-84% increased in specific gravity during storage at both 4.4° and 12.8°C (Heinze et al. 1952). At 90% relative humidity, specific gravity of tubers remained practically unchanged in storage up to 6 1/2 months at 4.4° and 10°C. Talley et al (1961) and Fitzpatrick et al (1964) found that specific gravity of tubers changed little in storage at 3.3°C, indicating that shrinkage was due to a loss of both solids and water in the ratio in the original composition. In general, specific gravity of potatoes increases with length of time in storage and indicates an increase in percentage of solids in the tubers (Terman et al. 1950). This is a result of greater loss of water by evaporation than loss of solids by respiration.

Dry matter : The main factors, which account for the increased dry matter losses during cold storage, are mechanical injuries of the tubers, sprouting, too low or too high a temperature and low relative humidity. The tubers with damaged skin and pulp as well as sprouting tubers respirate more intensely and this increases starch consumption for oxidation processes. The dry matter losses during cold storage of healthy, undamaged potato tubers at 5-6°C and relative humidity of 85-90% do not exceed 1%. However, Lisinska's (1989) investigations indicated that dry matter content in the tubers increased together with the maturity of the tubers and then decreased during the curing period, but remained practically constant during the storage period.

Sugar content : Sweetness of potatoes increases, when they are stored at comparatively low temperatures because of accumulation of reducing sugars and sucrose (Pushkarnath 1976). During storage at low temperatures, reducing sugars accumulate more rapidly than sucrose (Schwimmer et al. 1954). The amount of sugars formed during low-temperature storage depends on variety, maturity of tubers, pre-storage conditions, storage temperature and sprouting.

Van Vliet and Schriemer (1960) found different levels of sugar contents in different varieties grown under identical conditions after storing at 2°C. Samotus et al (1974) also reported that the sugar formation in a number of Polish varieties stored at 1°, 2° and 6°C for 18 to 22 weeks was correlated with varieties. Marwah (1997) reported that change in reducing sugar content during cold storage depended on variety of the potato. Peshin (2000) also found significant varietal differences in reducing sugar contents at storage temperature of 5-7°C among nine commonly grown Indian potato varieties (Table 5). Burton (1966) stated that apart from vatietal differences, maturity might be one of the principal factors affecting sugar content during cold storage. Freshly harvested immature tubers contain appreciable amounts of free sugars, mainly as sucrose. As the tuber matures, the sucrose percentage decreases and starch increases. At 0°C, the predominant accumulation is fructose,

TABLE 5. REDUCING SUGAR CONTENTS (mg/g FRESH WEIGHT) OF DIFFERENT INDIAN POTATO VARIETIES DURING STORAGE AT 5-7°C

Variety	Storage period, days			
	0	30	60	90
'Kufri Lauvkar'	2.9	3.2	3.9	5.0
'Kufri Badshah'	4.1	4.2	4.7	5.2
'Kufri Jawahar'	0.7	3.6	4.6	4.8
'Kufri Sutlej'	3.5	4.0	4.5	5.3
'Kufri Chandramukhi'	2.6	2.8	3.1	5.8
'Kufri Lalima'	2.9	3.2	4.1	4.4
'Kufri Kuber'	0.5	1.1	1.5	1.8
'Kufri Ashok'	1.0	2.7	4.0	4.2
'Kufri Bahar'	2.5	2.9	3.7	4.4

Peshin (2000)

but in mature tubers, sucrose predominates (Samotus and Schwimmer 1962).

The temperature to which the potatoes are exposed before cold storage may also affect the eventual variation of the sugar content (Es van and Hartmans 1989 b). Iritani and Weller (1976) showed that a great deal may go wrong in a 14 days period, preceding the commencement of final storage at low temperature. If the temperature during this period is higher or lower than 9 to 10°C, the level of reducing sugars will be much higher later on, in this case, after 6 months of cold storage at 5.6°C. At temperatures of 3.3° and 5.6°C, the increase in sugar is less and at 10° and 15.6°C, a slight reduction may occur. Most of the reducing sugars in tubers stored at 15.6°C are in the sprouts (Treadway et al. 1949). Hyde and Morrison (1964) found that storage at 4.4°C resulted in accumulation of reducing sugars. Burton (1969) found that the pattern of sugar accumulation of tubers at 2°C could vary with their age, when placed at this temperature. Gradual reduction in temperature to 2°C, in contrast to rapid cooling, resulted in marked reduction in the amount of sucrose accumulated.

Enzymes : As stated earlier that during cold storage of potatoes, starch is broken down by enzymes to form sugars. Starch can be broken down by either starch phosphorylase or by amylase. Potatoes contain low amylase activity and this enzyme is essentially inactive *in vitro* at 0°C. It appears to be a factor in starch degradation and sugar formation at low temperatures (Arreguin-Lozano and Bonner 1949). Storage time and temperature have no effect on amylose contents of starches and in potatoes or on their amylose-amylopectin ratio (Schwimmer et al. 1954). Phosphorylase is equally active in potatoes at all storage temperatures, but it does not attack starch in potatoes stored at high temperatures. Hyde and Morrison (1964) reported that phosphorylase activity was greater at 4.4°C than at 21.1°C. Apparently, this is due to the formation of an inhibitor of phosphorylase at higher temperatures that disappears at low storage temperatures.

Since phosphorylase catalyses the breakdown of starch, it may be a factor, influencing the sugar accumulation and dark colour of chips of potatoes stored at 4.4°C (Smith 1987). Conditioning of potatoes at room temperature after cold storage resulted in decreased phosphorylase activity as well as lower sugars and inorganic phosphate content.

Basal and total invertase activities increased in tubers stored at low temperatures, indicating increase in the synthesis of enzyme. However, in tubers stored at higher temperatures, the level of inhibitor was high, resulting in an increase in sucrose content (Nayer and Nand 1986). The effect of temperature and storage time on invertase activity and resultant sugar contents is shown in Table 6.

Weaver et al (1970, 1971) concluded from their studies using two natural potato substrates, chlorogenic acid and tyrosine, that phenolase did not show consistent relationships with blackspot susceptibility. Neither catalase nor peroxidase activity, nor any ratio of either catalase or peroxidase activity to monophenolase or polyphenolase activity, could be used for predicting a high probability of tissue resistance to blackspot. It may be, however, that catalase and peroxidase could directly

TABLE 6. INVERTASE ACTIVITY (MICROMOLE SUCROSE HYDROLYSED PER GRAM OF FRESH WEIGHT) AND SUGAR CONTENT (MG/100 G FRESH WEIGHT) IN POTATOES AT 3.5°C AND 30-32°C

Period of storage, days	3.5°C			30-32°C		
	Invertase activity	Reducing sugars	Sucrose	Invertase activity	Reducing sugars	Sucrose
0	10.1	314	166	–	–	–
25	13.9	850	246	6.5	297	342
50	16.9	1447	321	7.3	313	378
75	22.4	1530	442	6.6	354	854
100	25.8	1655	562	6.9	355	1323
125	29.3	1525	725	6.1	257	1806
150	26.6	1723	875	3.5	327	2423

Nayer and Nand (1986)

contribute to blackspot susceptibility.

Nitrogen compounds : The protein contents of potatoes are rather low, ranging from approximately 1.5 to 2.5% of the fresh weight. Storage conditions, specially temperature, may have an effect on the proportions of nitrogenous fractions in tubers, but total nitrogen content changes only slightly in cold storage (Fitzpatric and Porter 1966; Mazza 1983). Habib and Brown (1957) reported that cold storage slightly affected the amount of basic amino acids in tubers. The same authors earlier, (1956) found no significant differences in free amino nitrogen of tubers before and after storage at 4.4°C, but after several weeks of reconditioning at 23.9°C, the amount was greatly reduced.

Storage at 7.2°-10°C results in non-significant change in the acid content of tubers. Coleman and King (1984) examined the changes in proline content during cold storage and concluded that proline content increased with increase in storage temperature. Talley et al (1964) found that proline content increased during cold storage but other amino acids showed no consistent trend. Talley et al (1961) stated that the apparent inverse relationship between total and soluble nitrogen and total solids, when calculated on a moisture-free basis was due to the storage of other constituents, presumed to be principally starch, in the case of high-solid potatoes.

Organic acids : Sweeney et al (1969) found that citric acid increased and malic acid usually decreased in storage at 12.8°C. The potatoes of high specific gravity contain more citric acid and less pyroglutamic, malic and phosphoric acids than tubers of low specific gravity (Schwartz et al. 1961). The acids attain either their maximum or minimum concentration after one or two months of storage at 3.3°C. Changes in organic acids usually were smaller in potatoes stored at 12.8°C than in those at ordinary temperatures. Low temperature storage resulted in decreased pH and increased malic and citric acid contents in tubers. However, no significant relation was found between pH and content of malic as well as citric acid (Shekhar and Iritani 1979).

Ascorbic acid : The potato is considered to be the good source of vitamin C or ascorbic acid. During cold storage, however, ascorbic acid content of the tuber decreases. Storage at 5°C results in faster disappearance of ascorbic acid than

storage at 15°C (Rolf 1940). At temperatures as low as 0°C, the loss is more rapid than at 10°C (Karrika et al. 1944; Murphy 1946). However, ascorbic content increases when potatoes, previously held at 10°C or higher, are transferred to 0°, 1°, or 5°C (Kelly and Somers 1949). Heilinger and van Vilet (1960) reported that storage for 10 months at 18.3°C resulted in larger losses in ascorbic acid than storage at 1.1°C, 3.9°C, or 7.2°C. Ascorbic content decreased slightly as tubers approached maturity but decreased markedly during storage at 5°C and 10°C (Yamaguchi et al. 1960).

Factors affecting the storage losses

A number of factors such as physical, physiological, entomological, microbial, crop factors, etc. are responsible for the losses in potato cold storage. Effects of these factors have been discussed in the following sections.

Varieties : Very little information is at present available on the exact temperature needs for cold storage of different potato varieties in commercial use in the world. There are also no standards set by the trade about the regulation of temperatures in relation to variety. All the varieties are often stored in the same chamber under identical temperature ranges, with the result that heavy storage losses are often incurred. Therefore, need for adjustment of storage conditions, taking the variety into consideration, is of great and immediate importance. However, the potato varieties selected for longer storage should show some desirable characteristics like - long periods of dormancy after reaching maturity, moderate tendency to sprouting, limited intensity of vital processes in the tubers during storage and resistance to mechanical damage during harvesting and handling.

Agrotechnological treatments : Healthy and mechanically undamaged potatoes are suitable for storage as the losses of weight and sprouts are inconsiderable (Lisinska 1989). Therefore, during the growing period, a specific number of treatments should be applied in adequate amounts and time in order to obtain potato tubers suitable for storage. Healthy seed potatoes are treated with fungicides and bactericides, sprouted and planted in the field early enough to allow mature potato tubers to be obtained comparatively earlier. Thus, their harvesting takes place at proper temperature and moisture levels of the soil. Adequate fertilization can affect the potato maturation at the time desired and also the increased resistance of potatoes to conditions that may damage the crop. High nitrogen levels can delay maturation and deteriorate resistance to mechanical damage, unless they are enriched with sufficient amounts of phosphorus and potassium. During the growing season, special attention should be paid to weed control and the control of fungal and bacterial diseases. The control of fungal and bacterial diseases during the growing season protects the tubers from such diseases and hence, the crop is healthy and this guarantees good storage conditions.

Maturity of tubers : Losses are slightly higher, when immature tubers are held in cold storage, particularly in the first month (Table 7). This may be attributed to (i) high respiratory activity, particularly soon after harvest (ii) rapid transpiratory loss of water due to incomplete curing of the skin in early stages and (iii) high percentage of tubers with skin bruises

Harvesting and post-harvest handling : If potatoes are to be stored for a relatively long period (3-7 months), allowance should be made for this at the time of harvesting and during post-harvest handling (Sparenberg 1989). Before harvesting, it is important to allow the tubers to set as far as possible in the ground. The tuber then has a chance to form a cork layer, which protects it against damage during lifting and transport. It is also important for the harvested potatoes to be exposed as little as possible to the sun so that the tuber temperature does not rise too high (which might cause black heart). Harvesting should, therefore, preferably be carried out in the morning and not in the middle of the day. The potatoes must then be placed in the shade as quickly as possible and preferably protected from direct sunlight during transport. Since ageing and deterioration of potatoes continue after harvest, the marketable life depends greatly on the temperature and care in physical handling. The effect of rough handling of potatoes is cumulative. Mechanical damage allows increased moisture loss. Therefore, harvesting and post-harvest handling of the potatoes destined for cold storage should be carried out carefully.

Condition of tubers at the time of storage : Clean tubers with well-suberized skin keep better than unclean and otherwise damaged tubers. Several saprophytic organisms can invade the tuber and thus be a cause for heavy rottage. Clean and well-graded tubers of an uniform size keep better under cold storage and at the end of the storage period, yield attractive and good-sized seed material for planting.

Pre-cold storage treatment : It is necessary to ensure that seed tubers to be stored in the cold storage receive proper pre-cold storage treatment. Instances are not lacking, when stocks harvested in the field have to rot even at low temperature in the cold storage. The percentage of storage losses (at the end of 12 weeks of storage) in stocks brought at different intervals after the harvest date and stored at two temperatures showed marked variations (Table 8).

TABLE 7. COLD STORAGE LOSSES AT 1.28°C

Period of storage, months	Percentage loss	
	Mature	Immature
1	1.09	2.50
3	2.91	3.36
5	3.78	4.57
7	5.11	5.86

Pushkarnath (1976)

TABLE 8. ROTTAGE OF POTATO TUBERS WHEN STORED AT VARYING INTERVALS BETWEEN HARVEST AND COLD STORAGE

Interval between harvest and storage, days	Percentage loss at	
	7°C	4.4°C
1	28.3	2.0
3	17.3	1.4
7	8.5	0.7
14	2.7	0.6
21	1.5	0.3

Pushkarnath (1976)

Quick transfer of stocks from low to high temperatures may lead to physiological breakdown of the tuber. To overcome this problem, a short period of pre-cold storage treatment at somewhat higher temperatures, about 15°C, before the transfer of material to low temperature storage chambers is a desirable practice. The pre-cold storage treatments enable the potatoes to acclimatise with the low temperature that exists in the cold storage chamber itself. It was, however, found that the general practice was to load the sealed potato bags directly in the cold chambers without keeping the tubers in the pre-cooling chamber for sufficient period (Anon 1964).

Convective heat transport : In cold storage, the potatoes are packed in gunny bags and these bags are arranged in stacks. The potato near the surface of the stack is subjected to rapid cooling than inner most part of the product owing to the resulting difference of temperature in the stack (Beukema et al. 1983). This temperature difference within the stack produces convection currents owing to the resulting difference of density. These convective currents are responsible for additional temperature reduction of the potatoes.

Metabolic heat of respiration

It has already been discussed that stored potatoes generate heat by respiration. The amount of heat generated is not constant throughout the storage period. It also differs from variety to variety. Burton et al (1955) have stated that, if stored potatoes are producing 11.73 W/tonne, an average value in the middle of the season, this is sufficient to raise their temperature by about 0.27°C per day. The metabolic heat of respiration of potatoes is affected by the following -

Effect of post-harvest management : As the end quality of potatoes destined for cold storage has a direct effect on respiratory activity, it is mandatory to have a proper harvesting and post-harvest management in order to reduce the losses. Potatoes that are carefully handled to avoid bruising during harvest and storage respire at a lower rate than potatoes that are handled less carefully (Smith 1933). The same author found a large increase in respiration rate of potatoes on the second or third day after wounding, followed by a gradual return to the normal rate. Some of this apparent increase in rate of respiration, however, may be due to facilitating the escape of carbon dioxide by injury to the periderm of the potato.

Effect of sprouting : Effect of sprouting is insignificant during the first three weeks of growth, when the sprouts are very small and thereafter, the respiration rate increases. When the tuber has about 1% of its own weight of sprout, respiration has increased by 50% (Burton et al. 1955). However, use of sprout inhibitors reduces the rate of respiration. Respiration rate of potatoes that receive doses of 5, 15, and 25 kilo-REP of gamma radiation decreased about 30%, the first day following treatment. The second day, it increased to 60% above the untreated tubers. This was followed by a decline until during the thirteenth week of storage, the irradiated tubers respired about 5% less than the untreated ones (Brownell et al. 1957).

Degree of maturity : The degree of maturity of the potatoes has a marked effect upon their rate of respiration and consequently heat production. It was found that, immature

TABLE 9. HEAT OF RESPIRATION (MILLI WATT/KG)

Maturity	Temperature 5°C	10°C	15°C	20°C
Potatoes, immature	34.9	41.7 – 62.1	41.7 – 91.7	53.8 – 133.7
Potatoes, mature	17.5 – 20.4	19.7 – 29.6	19.7 – 34.9	19.7 – 47.0
Potatoes, very mature	15.0 – 20.4	20.0	20.4 – 29.6	27.2 – 35.4

Burton et al (1955)

TABLE 10. RESPIRATION HEAT PRODUCTION OF POTATOES STORED AT DIFFERENT TEMPERATURES

Storage temperature, °C	0	4	6	8	10	14	20
Respiration heat, W/tonne	20	18	19	21	22	25	31

Van't Ooster (1999)

potatoes respire nearly 70% more rapidly immediately after lifting than mature tubers similarly lifted and stored at the same temperature (Burton 1955). This may be due to a large content of sugars, especially saccharose, which is regarded as a respiratory substrate. After 11 days, however, the rate of respiration of the immature tubers had fallen almost to the level, which had by then dropped to a fairly steady value to that of the mature tubers. The effect of maturity is shown in Table 9.

Effect of temperature : The metabolic heat of respiration is strongly influenced by temperature of potato. Respiration rate of potatoes increases with increase in temperature as shown in Table 10. The effect of temperature upon respiration is at least two-fold. At any one sugar content, a decrease in temperature will lead to a decrease in the rate of respiration, but over the lower range of storage temperatures, a decrease in temperature will also lead to an increased content of sugar. Decreasing the storage temperature below 10°C will, therefore, have two opposite effects - a tendency to a decrease in respiration rate because of low temperature and a tendency to an increased respiration rate because of the greater content of the sugar. The net result is that there is a minimum rate of respiration at about 4.4–7.2°C. Hopkins (1924) showed that the minimum rate of respiration of potato tubers occurred at a temperature of 3°C and that the rate of respiration of tubers stored at 0°C was greater than that of tubers stored at 4°C. According to Paez and Hultin (1970), tubers placed at 21.1°C after initial storage for 36 days at lower temperatures increased in respiration, which was inversely proportional to the previous storage temperature. Burton (1974) showed that sudden change in storage temperature caused 'respiratory burst' in the tuber.

Storage period : Burton (1963) noted a tendency for tubers to have a Respiration Quotient (RQ) higher than 1.0 during the first week of storage and as high as 2-4 after prolonged storage accompanied by sprout growth. During the main storage period, however, he found that the RQ was close to 1.0.

Storage atmosphere : In freshly harvested mature tubers, a fall in the oxygen concentration of the surrounding atmosphere

from 20.9 to 3% causes only 20-30% decline in respiration rate. A further decrease in the oxygen content of the atmosphere below 3%, however, results in a rapid, more or less linear, decline in respiration rate. However, an increase in carbon dioxide concentration up to 5-7% can completely inhibit the respiration rate (Es van and Hartmans 1989 c).

Stimulatory and inhibitory substances in tuber : Substances such as malonic acid, ethylene, HCN, azide, antimycin, etc. are known to effect respiration. Ethylene and cyanide stimulate respiration and in potato tubers, are associated with an enhancement of glycolysis and an increase in sucrose accumulation (Es van and Hartmans 1989c).

Prediction of metabolic heat of respiration : Different equations have been proposed to predict the heat of respiration at different temperatures. Few of them are listed below -

(i) According to Burton et al (1955), the amount of metabolic heat (in B.T.U.) produced by a tonne of respirating potatoes in 24 h may be obtained by multiplying the number of milligrams of carbon dioxide produced per kg per hour by 250.

(ii) Heat of respiration after some days of harvesting is linearly proportional to rise in temperature (Bird 1976),

$$Q = 6.99\ (T_p) - 17.7 \qquad \text{J/kg-hr}$$

where,

T_p = temperature of potato, °C

(iii) The rate of respiration in milligrams carbon dioxide per kg tuber fresh weight per hour (Schippers 1977)

$$= \frac{\%\ CO_2 \times 0.01 \times (4250 - \text{volume of tubers in ml}) \times 1.85}{\text{No. of hours} \times \text{kg of tubers}}$$

The factor 1.85 in the formula is to convert the millilitres carbon dioxide into milligrams.

(iv) Beukema and Bruin (1982) found that heat generation was temperature dependent of Arrhenenious type, i.e.,

$$Q = Q_o\ e^{-(E/T)}$$

The product dependent variable Q_o and E should be derived from measured values of the rate of heat generation at different temperatures.

(v) The carbon dioxide production of potato can be expressed as a function of temperature, by a least-square regression fit of the form (De Baerdemaeker et al. 1999).

$$m_{CO2} = 0.01709\ (9\ T_p/5 + 32)^{1.769}$$

where, m_{CO2} = the carbon dioxide production per unit mass of a product (mg/kg/h), T_p = the mass average product temperature in °C. Then, the rate of heat generation by respiration (in J/kg/hr) can be obtained by -

$$Q = 10.7\ m_{CO2}$$

(vi) Heat production (in W/m^3) of the potatoes in storage can be estimated with the following linearized function (Van't Ooster 1999)

$$Q = 0.4\ T_p + 3.4\ (T_p - \text{Potato temperature in °C})$$

This is valid in the temperature range from 3 to 10°C.

Heat conduction : As it has already been stated that in the beginning of cooling process of packed bag, heat is transferred by conduction from within the product. The packed bags of potatoes behave as a porous medium, and the thermal conductivity of the packed bag of potatoes is less than only of potatoes themselves. If the bags are packed tightly, the porosity of the bags will be less and this will facilitate faster cooling of the potatoes.

Storage conditions : The storage temperature, relative humidity and air velocity in the storage chamber affect the storage losses to a great extent. Temperature is the principal factor affecting the quality of potatoes and losses during storage (Rastovski 1989 b). The imbalance in storage temperatures may make stored potatoes unfit for consumption due to physiological breakdown of the tuber, sprouting, freezing injury, fungal rottage, undesirable changes in chemical composition, etc. 'Kufri Kuber' is very sensitive to small variations of storage temperatures and often suffers more in cold storage (Pushkarnath 1976). The optimum storage temperature depends on the quality of the potatoes, as they are loaded into the store as well as on the eventual purpose for which the potatos are to be used. However, the temperatures given in Table 11, may be specified as a general guide for long-term storage of potatoes (Rastovski 1989 c).

The relative humidity below 80% leads to the drying of the potato tubers. Dampness, on the other hand, favours the growth of microorganisms, causing mould and rotting of the potatoes. A favourable value for the relative humidity of the storage atmosphere is 95% or more. Forced convection cooling of the potato bags is desirable over natural convection. An increase in storage velocity up to 0.75 m/s significantly lowers the cool-down time, but there is negligible effect on cool-down time after 3.0 m/s (Chourasia et al. 1999).

Diseases and defects : Potatoes may be affected by fungus or bacterial diseases, which may spread extensively in appropriate conditions, causing substantially losses (both qualitative as well quantitative) during cold storage. Storage diseases are basically the after-effects of infections of tubers with microorganisms, which take place either in the field during the plant growth or in the harvest and post-harvest phases. Thus, the microorganism is either present inside the tuber or is carried on its surface. Bruises or cuts during harvesting, riddling, bagging and transport of tubers readily provide avenues for the entry of the microorganisms. Therefore, care should be taken during harvesting and post-harvest handling to avoid mechanical injuries in order to reduce the tuber decay during cold storage.

TABLE 11. RECOMMENDED STORAGE TEMPERATURES FOR DIFFERENT USES OF POTATOES

Use of potatoes	Storage temperature
Seed potatoes	2–4°C
Potatoes for fresh consumption	4–7°C
Potatoes for chipping	7–10°C
Potatoes for French fries	5–8°C
Potatoes for granulation (mashed potatoes)	5–7°C

Rastovski (1989 c)

Stack dimensions : Burton et al (1955) have shown experimentally that the peripheral cooling ceases to affect the temperature beyond a certain distance from the surface and increase in horizontal dimensions beyond this has no effect on the temperature of the potatoes. This means that the centre-most potatoes of large stack dimensions are more susceptible to rotting. In line with these results, Chourasia et al (1999) have concluded that for the same volume of bag, long rectangular bag is preferable over cubic one. Also, the outside surfaces of the outermost tubers were close to the temperature of the store air. This indicates that if the storage air temperature remains below the 4°C for rest of the storage period, the cold injury to the potatoes results (Burton et al. 1955).

Stacking arrangement and packaging : In cold storage, the potato bags are stacked in a bulk on wooden platform having slots on it. The potatoe bags are loaded in haphazardous manner, leaving very less space for air circulation around the stacks, which would increases the cool-down time, thus causing various losses during storage (Anon 1999). Generally, 3.4 m^3 of volume is required per tonne of potato to be stored (Prasad 1995). Other than the stacking pattern, the packing of potato also affects the losses to some extent. Tubers stored in open trays at low temperature lost about 10% of their weight in 9 months, but those stored in gunny bags lost only 5% weight in the same storage period.

Conclusion

It may be concluded from the above discussion that the potato, storage conditions and storage duration are the three main variables that determine the storage losses. Apart from these, stack dimensions, stacking arrangement and operation as well as management of cold storage also affect the storage losses to a great extent. Also, the storage conditions required to limit different forms of storage losses are mutually exclusive. Hence, the optimum storage conditions will have to be a compromise between these conflicting requirements for the relevant situation.

Low storage temperature is necessary to avoid onset of rotting at the centre, but it should be high enough to avoid cold injury on the potatoes at the edges. Refrigeration system and air velocity decide this temperature. Air velocity should be high enough to give a reasonable heat transfer coefficient, at the same time, should be low enough to avoid any excessive expenditure of energy. Low stack-width offers less resistance to heat transfer for the centre-most section of the bag to get cooled, so that cool-down time is reasonable. But it should be high enough for economical loading of the potato in the cold store because the revenue earned by the cold store, is proportionate to the quantities of potatoes loaded. Further, stacking should be such that it does not obstruct airflow around the bags and should not touch the floor and walls directly.

To avoid quick loading and overloading of storage chambers, it is best to keep tubers in pre-cooling chambers for a week or ten days before they are transferred to cold storage chambers. As different potato varieties are stored in the same chamber under identical storage conditions, this results in heavy storage losses. Therefore, need for identifying the suitable storage conditions, taking the variety into consideration, is of great and immediate importance.

Careful handling of potatoes to minimize the mechanical injuries at the time of harvest and post-lifting helps in avoiding the losses in cold storage to a great extent. Quality maintenance in cold storage is added by harvesting the potatoes at optimum maturity or quality, rapid handling to minimize deterioration, enforcing good plant sanitation procedure, providing high relative humidity to minimize moisture loss and ensuring proper refrigeration throughout the storage period. Finally, experienced and trained personnel should be engaged in operation and management of cold storage.

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Effect of Hydrocolloids on the Rheology of Tamarind Sauce

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The effects of three different hydrocolloids guar gum, sodium alginate and gum acacia on the flow behaviour properties, serum loss and flow value of tamarind sauce were studied. The hydrocolloids were added to the sauce at different concentrations (0 to 1.5%) and rheology was studied at different temperatures (20 to 70°C). The power law and Arrhenius equations were used to determine the consistency index, flow behaviour index, Activation energy and arrhenius constant. Multiple regression equations were fitted to obtain the consistency index and apparent viscosity. The activation energy of flow for the hydrocolloid containing tamarind sauce samples varied from 2.687 to 23.101 kJ/gmol. Guar gum was found to give maximum consistency index, lower flow values and least serum loss followed by sodium alginate and gum acacia.

Keywords : Tamarind sauce, Hydrocolloids, Consistency index, Arrhenius constant, Serum loss, Flow value.

The tamarind tree is a native of Africa and Tropical Asia and is grown for its pulpy fruit tamarind (*Tamarindicus indica*), which has a sweet sour taste. India is one of the major tropical countries, producing and exporting tamarind (fresh and dry) and tamarind juice concentrate. The dehydrated fruit is used in *chutneys, curries* and sauces. Tamarind sauce is prepared from tamarind pulp by adding sugar, salt, spices etc. and then heating the sauce to the desired consistency. Hydrocolloids can be added to increase the viscosity of the sauce at a lower total solids content. The flow properties of a large number of liquid foods have been reported (Rao 1977). The physical properties of tamarind juice concentrate at different temperatures and concentrations have been investigated by Manohar et al (1991). However, studies on the effect of hydrocolloids on the rheology of tamarind sauce has not been investigated. Hydrocolloids are polysaccharides composed of simple sugar building units (Sanderson 1981; Glicksman 1969, 1982; Whistler and BeMiller 1993) and are widely used in the food industry as gelling, stabilizing, thickening and suspending agents. All of them have hydrophillic molecules, which combine with water to form viscous solutions. Rheological properties are required to design and evaluate food processing equipment such as pumps, heat exchangers and evaporators. The present investigation was carried out to study the effect of different hydrocolloids on the consistency index, serum loss and flow value of tamarind sauce at different concentrations and temperatures.

Materials and Methods

Preparation of tamarind sauce : Tamarind (normal variety stored in gunny bags at ambient conditions) was procured from the local marked and was soaked in water at 80°C for 30 min. The hydrated tamarind was passed through a laboratory pulper (Narang Corporation, New Delhi) to get tamarind pulp with 10% TSS. The tamarind sauce was prepared by mixing 5% sugar, 0.5% salt and 0.1% sodium benzoate to the pulp. The final TSS of the sauce was 16% and pH was 2.9. Three different hydrocolloids viz., guar gum, sodium alginate and gum acacia (CDH Chemicals, Mumbai) were added to the tamarind sauce at levels of 0, 0.5, 0.75, 1.0, 1.25 and 1.5%. The hydrocolloids were pre-blended with sugar and salt and then added to the sauce during the final stages of cooking. The sauce was filled in glass bottles sealed with crown corks and stored at 5°C, till further study.

Rheology : Rheological properties were determined using Brookfield Viscometer (Brookfield Engineering Inc. model DV-II). A 500 ml beaker with a diameter of 8.5 cm was filled with the tamarind sauce sample to a height of 8.5 cm and brought to the desired temperature in the TC-500 water bath (Brookfield Engineering Inc.) Measurements were taken after the spindle (no. 14) was immersed for 2 min so as to allow thermal equilibrium in the sample and to eliminate the effect of immediate time dependence. Log-log plots of shear stress v/s shear rate were plotted to determine the consistency index (k) and flow behaviour index (n), using the power law equation (Clark 1978 ; Holdsworth 1971 ; Rao 1977).

$$t = k\ (dv/dy)^n$$

where t = shear stress (Nm^{-2}), k = consistency index (Nsm^{-2}) and dv/dy = shear rate (s^{-1}).

The effect of temperature on the consistency of tamarind sauce can be described by the Arrhenius relationship (Saravacos1970 ; Rao et al. 1984).

$$k = ko \exp (E_a/RT)$$

where ko = Arrhenius constant, E_a = Activation energy (kJ/gmol) of flow, R = gas constant and T = absolute temperature in Kelvin.

Regression analysis was carried out using Minitab Statistical Software (Minitab Inc. USA) to compute second order polynomials. The shear rate, temperature and hydrocolloid concentration were taken as independent variables and they had values of X_1 (shear rate) 0.8, 2.0, 4.0, 20.0 and 40.0 s^{-1}, X_2 (temperature) 20, 30, 40, 50, 60 and 70°C and X_3 (hydrocolloid concentration) 0, 0.5, 0.75, 1.0, 1.25 and 1.5%. The polynomials were fitted to measure the apparent viscosity (μ). The following equation was used.

$$\ln \mu = a + \sum_{j=1}^{3} b_j X_j + \sum_{j=1}^{3} c_j X_j^2 + dX_1X_2X_3 + eX_1^2X_2^2X_3^2 + fX_1X_2 + gX_1X_3 + hX_2X_3 \quad ....(1)$$

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Similarly, a second order multiple interaction was used to express consistency index as a function of two independent variables, which were Z_1 (temperature) 20, 30, 40, 50, 60 and 70° C and Z_2 (hydrocolloid concentration) 0, 0.5, 0.75, 1.0, 1.25 and 1.5%. The following equation was used.

$$\ln k = a + \sum_{j=2}^{2} b_j Z_j + \sum_{j=2}^{2} c_j Z_j^2 + dZ_1Z_2 + eZ_1^2Z_2^2 \quad \text{.....(2)}$$

Flow value : The consistency of the sauce at a temperature of 25°C was also measured using an instrument similar to the USDA consistometer (Bourne 1982). It consisted of a flow sheet made of glass over which the product was made to flow. The receptacle holding the sauce was a PVC cylinder having an inner diameter of 4.5 cm and a height of 10 cm. The cylinder was filled with the sauce and then raised upwards to allow the sauce to flow over the glass sheet. The distance of flow after 1 min was measured from the center of the cylinder in centimeters. The flow value was taken as the average of the reading at four quadrants of the flow sheet and expressed in centimeters. The readings were taken at the edge of the sauce and did not include any serum that exuded from the sauce.

Serum loss : The ability of the gums to hold water in the sauce was also measured. Sauce (20 g) was taken in a centrifuge tube and then centrifuged at 5000 rpm for 10 min. The supernatant was discarded and the remaining sauce was weighed.

$$\% \text{ serum loss} = \frac{\text{weight of serum removed} \times 100}{\text{weight of sauce}}$$

TABLE 1. EFFECT OF TEMPERATURE ON THE CONSISTENCY INDEX OF TAMARIND SAUCE

Temperature, °C	Consistency coefficient, Nsm^{-2}	Flow behaviour index, n	r^2
20	3.1174	0.3618	0.917
30	2.9661	0.3106	0.926
40	2.61	0.2880	0.928
50	2.27	0.2927	0.908
60	1.58	0.2837	0.916
70	1.19	0.3591	0.920
Coeff. variation (%) (n)		11.2220	

Results and Discussion

Consistency index : The consistency index of tamarind sauce decreased with increasing temperature (Table 1). Addition of hydrocolloids led to an increase in the consistency index of the sauce and the consistency index increased with the increase in hydrocolloid concentration (Table 2). The magnitude of regression coefficients of k indicated that the power law fitted adequately well with the rheological data. The

TABLE 2. EFFECT OF HYDROCOLLOID CONCENTRATION AND TEMPERATURE ON THE CONSISTENCY INDEX OF TAMARIND SAUCE

Guar gum		0.50 %			0.75 %			1.00 %			1.25 %			1.50 %	
°C	k	n	r^2	k	n	r^2	k	n	r^2	k	n	r^2	k	n	r^2
20	21.547	0.1799	0.987	47.456	0.179	0.980	80.631	0.130	0.984	98.948	0.0868	0.985	120.948	0.1124	0.977
30	17.623	0.2084	0.992	42.825	0.187	0.989	70.032	0.159	0.981	80.816	0.1440	0.989	120.700	0.1270	0.980
40	16.010	0.2108	0.992	40.841	0.192	0.975	59.689	0.184	0.968	75.422	0.1530	0.967	118.010	0.1055	0.966
50	13.696	0.2270	0.982	38.106	0.199	0.972	55.050	0.195	0.964	72.962	0.1691	0.961	116.760	0.1182	0.976
60	13.267	0.2210	0.980	35.212	0.212	0.967	50.176	0.212	0.952	70.210	0.1701	0.969	106.930	0.1460	0.977
70	11.926	0.2520	0.985	28.477	0.248	0.963	44.228	0.235	0.954	63.826	0.2040	0.956	103.609	0.1544	0.959
Coeff. variation (%)		10.984			12.203			20.100			25.238			15.174	
Sodium alginate		0.50 %			0.75 %			1.00 %			1.25 %			1.50 %	
20	6.96	0.345	0.960	8.05	0.288	0.983	9.13	0.254	0.879	12.09	0.290	0.937	22.10	0.3047	0.955
30	6.36	0.279	0.862	7.44	0.323	0.906	8.16	0.279	0.923	9.27	0.316	0.936	21.55	0.2810	0.983
40	6.01	0.263	0.877	6.49	0.268	0.887	6.87	0.249	0.863	8.27	0.345	0.961	14.79	0.3410	0.986
50	5.28	0.242	0.904	5.77	0.291	0.967	6.10	0.290	0.963	7.50	0.360	0.979	11.27	0.3530	0.989
60	4.53	0.241	0.916	4.99	0.252	0.887	5.81	0.401	0.943	6.90	0.385	0.983	10.79	0.3309	0.955
70	3.56	0.260	0.927	3.96	0.264	0.920	4.10	0.348	0.961	5.50	0.394	0.986	9.65	0.3402	0.984
Coeff. variation (%)		14.248			9.013			19.598			14.465			8.314	
Gum acacia		0.50 %			0.75 %			1.00 %			1.25 %			1.50 %	
20	3.44	0.384	0.928	4.25	0.348	0.920	6.703	0.224	0.873	12.03	0.209	0.978	14.5	0.167	0.899
30	3.10	0.274	0.861	3.40	0.325	0.895	3.520	0.349	0.931	8.54	0.242	0.900	12.1	0.310	0.899
40	2.70	0.313	0.877	2.73	0.321	0.911	3.022	0.307	0.904	4.85	0.288	0.937	7.5	0.338	0.930
50	2.47	0.257	0.902	2.52	0.341	0.846	2.685	0.277	0.855	4.10	0.131	0.880	5.2	0.284	0.868
60	1.86	0.276	0.906	2.26	0.251	0.906	2.530	0.308	0.903	3.70	0.278	0.815	5.2	0.332	0.925
70	1.70	0.263	0.856	2.10	0.234	0.804	2.300	0.289	0.904	3.05	0.271	0.905	4.3	0.281	0.896
Coeff. variation (%)		16.294			15.966			14.176			25.001			21.939	

k (Nsm^{-2}), consistency index; n (dimensionless), flow behaviour index

addition of guar gum gave the maximum viscosity, followed by sodium alginate and then gum acacia. Fig. 1, 2 and 3 show the apparent viscosity of the sauce at different hydrocolloid concentrations and temperatures, when the shear rate was 4 s^{-1}. The viscosity increased with an increase in concentration and decreased with increase in temperature.

Guar gum has a straight chain of D-galactose - D-mannoglycan with many single galactose branches. Therefore it combines properties of linear and branched polysaccharides and has a higher molecular weight than the other two hydrocolloids (DeMan 1968). This explains the higher viscosity imparted by guar gum. Sodium alginate gave lower viscosity than guar gum. Alginate is a linear 1, 4-linked polyuronan consisting of β-D-mannuronic and α-L-guluronic acids. The insoluble alginic acid precipitates at pH <3.5 (DeMan 1968). The low viscosity imparted by sodium alginate could have been due to the precipitation of the alginic acid molecules at the low pH. Gum acacia gave the least viscosity in the sauce. It is considered to be a complex, highly branched, globular molecule, which is closely packed rather than linear and this may account for its low viscosity (Anderson and Dea 1971). Gum acacia solutions at low concentration are essentially Newtonian in behaviour and have very low viscosities compared to other polysaccharides of similar molecular mass (Garti and Reichman 1993).

Values for flow behaviour indices were less than 1 for all samples studied and this indicated that the sauce was pseudoplastic in nature (Tables 1 and 2). The value of n showed no definite trends with hydrocolloid concentration and temperature. Holdsworth (1971) and Steffe et al (1983) reported similar results regarding flow behaviour index. Increasing the temperature reduced the consistency index of the sauce in all cases. The decrease in consistency of the sauce with increase in temperature was maximum in case of gum acacia, followed by sodium alginate and least in case of guar gum. Changing the hydrocolloid concentration affected the activation energy of flow, especially in guar gum (Table 3). The change in activation energy was maximum in case of guar gum, followed by gum acacia and then sodium alginate. The relationship between consistency index and increasing hydrocolloid concentration was linear, but there was no linear relationship between the activation energy with increasing hydrocolloid concentration. The coefficient of variation for the Arrhenius constant was maximum in case of guar gum (Table 3).

Guar gum concentration most significantly affected the consistency index of the sauce both in linear and square terms. Sodium alginate concentration and sauce temperature significantly affected consistency index in linear terms. Gum acacia concentration in square terms and sauce temperature in linear terms affected consistency index most significantly (Table 4). The coefficients of regression equation obtained to predict apparent viscosity revealed that shear rate had the most pronounced effect on apparent viscosity both in linear and square terms, followed by hydrocolloid concentration (Table 5). The R^2 values in Tables 4 and 5 indicate that the coefficients of the regression equations can be used to predict the apparent viscosity and consistency index.

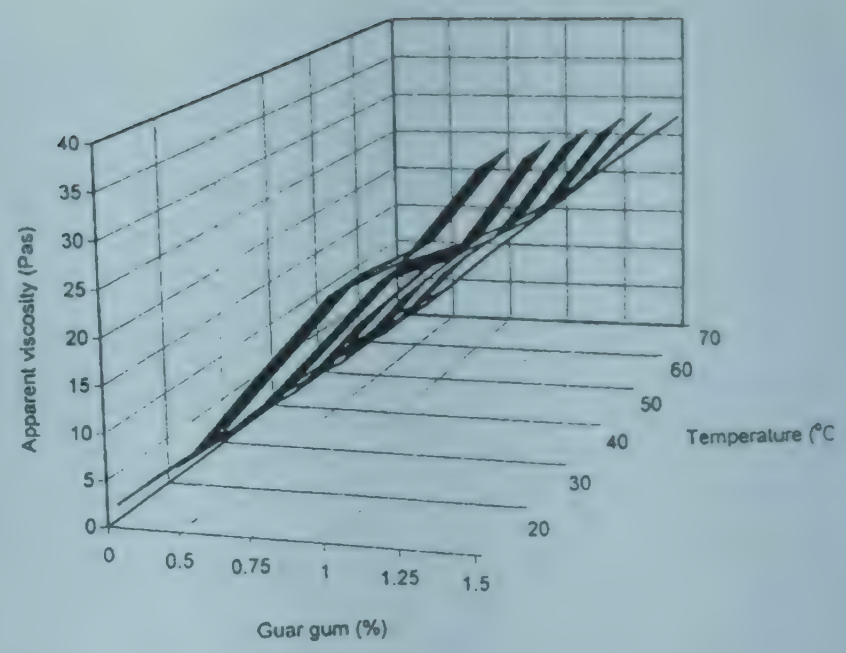

Fig. 1. Apparent viscosity of guar gum at (shear rate 4/s) different concentrations and temperatures

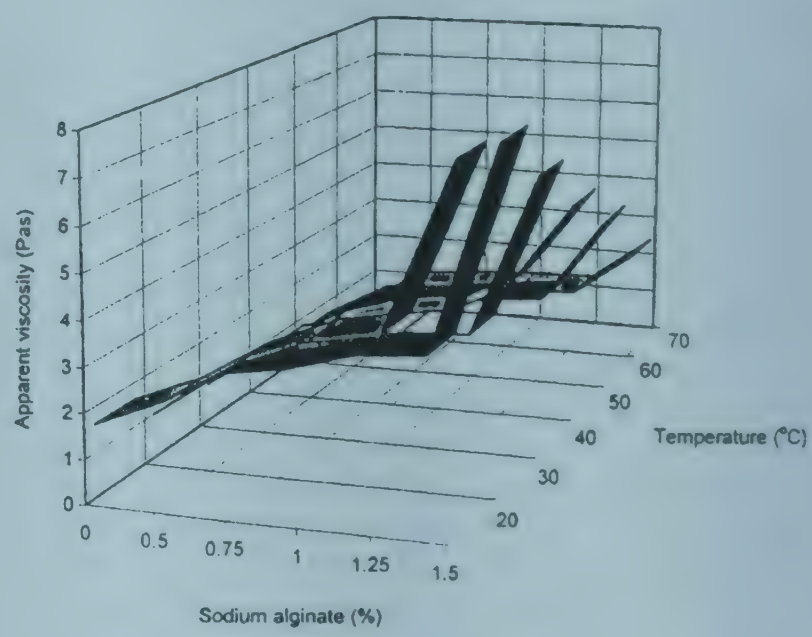

Fig. 2. Apparent viscosity of sodium alginate at (shear rate 4/s) different concentrations and temperatures

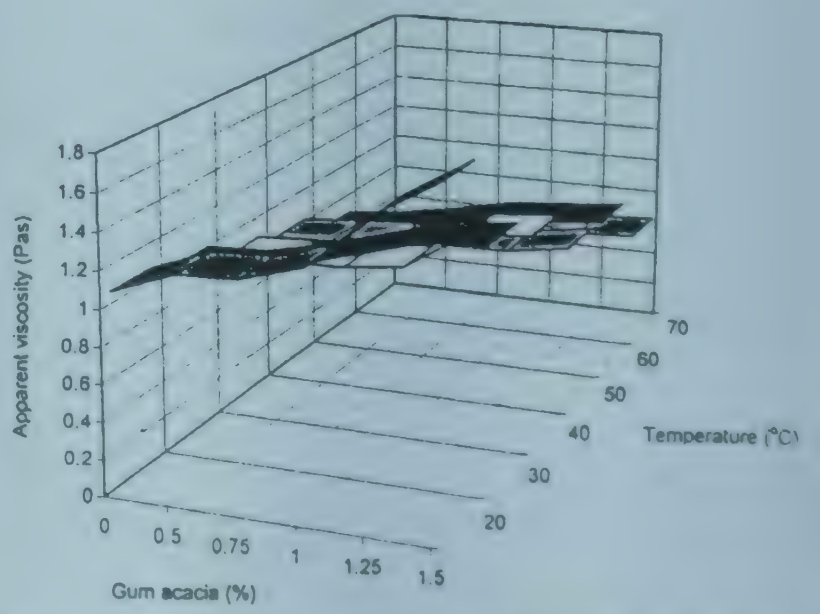

Fig. 3. Apparent viscosity of gum acacia at (shear rate 4/s) different concentrations and temperatures

TABLE 3. EFFECT OF HYDROCOLLOID CONCENTRATION ON THE ACTIVATION ENERGY OF TAMARIND SAUCE

Concentration	Guar gum			Sodium alginate			Guma acacia		
	E_a(kJ/gmol)	ko (Pas^n)	r^2	E_a(kJ/gmol)	ko (Pas^n)	r^2	E_a(kJ/gmol)	ko (Pas^n)	r^2
0.0 %	16.1197	0.00481	0.892	16.119	0.00481	0.892	16.1197	0.00481	0.892
0.50 %	9.5805	0.4074	0.977	10.644	0.09422	0.922	12.2620	0.02356	0.965
0.75 %	7.6373	2.122	0.923	11.54009	0.0758	0.958	11.6705	0.033	0.964
1.00 %	9.7883	1.439	0.993	12.232	0.0629	0.935	15.838	0.0079	0.829
1.25 %	6.4160	6.707	0.920	11.821	0.0908	0.970	23.101	0.000844	0.949
1.50 %	2.6870	41.272	0.832	15.563	0.03906	0.9303	21.142	0.0024	0.974
Coeff. variation (%)		186.61			55.88			108.53	

E_a, activation energy; ko, Arrhenius constant

TABLE 4. COEFFICIENT OF REGRESSION MODELS FOR CONSISTENCY INDEX

Term	Guar gum	Sodium alginate	Gum acacia
Constant	1.3724	1.5503	1.8874
Z_1 (b_1)	-0.0113*	-0.0131*	-0.0353***
Z_2 (b_2)	4.564****	1.0394*	-0.2731
Z_1Z_2 (d)	0.00452*	0.0049	0.00142
Z_1^2 (c_1)	-0.00005	-0.000044	0.000212*
Z_2^2 (c_2)	-1.503****	-0.0503	0.8215****
$Z_1^2Z_2^2$ (e)	0.000019	-0.000029	-0.000056*
R^2	99.4 %	91.5 %	95.1 %

Z_1 temperature (°C), Z_2 hydrocolloid concentration (%)

*p < 0.05, **p < 0.05, ***p < 0.005, **** p < 0.0005

TABLE 5. COEFFICIENT OF REGRESSION MODELS FOR APPARENT VISCOSITY

Term	Guar gum	Sodium alginate	Gum acacia
Constant (a)	1.483	1.7014	1.8805
X_1 (b_1)	-0.1783****	-0.1709****	-0.1944****
X_2 (b_2)	-0.0141*	-0.0218**	-0.0289**
X_3(b_3)	4.094****	1.158****	0.3701*
$X_1X_2X_3$	0.000087	0.00014	0.000072
X_1^2 (c_1)	0.0029****	0.0028****	0.0033****
X_2^2 (c_2)	-0.000034	0.000052	0.00011*
X_3^2(c_3)	-1.331****	0.0212	0.3869**
$X_1^2X_2^2X_3^2$ (e)	-3.5^{-8}* x 10^{-8}	-3 x 10^{-9}	2.36 x 10^{-9}
X_1X_2 (f)	0.000055	-0.000043	-5 x 10^{-7}
X_1X_3 (g)	-0.015*	-0.0071	-0.0062
X_2X_3 (h)	0.009**	-0.0024	-0.0045*
R^2	96.6 %	86.6 %	81.6 %

Z_1 shear rate (sec^{-1}), X_2 temperature (°C), X_3 hydrocolloid concentration (%)

*p < 0.05, **p < 0.05, ***p < 0.005, ****p < 0.0005

There was 82.18% increase in consistency index of sauce due to addition of guar gum from 0.5 to 1.5% at 20°C, which was maximum, when compared to the other two gums. The consistency index of the sauce by the addition of gum acacia decreased by 50% at 0.5% concentration and 70.03% at 1.5% concentration, when the temperature of the sauce was increased from 20 to 70°C. This higher decrease in consistency at 70°C could be attributed to the presence of a small amount of protein as an integral part of its molecule. Also, the gum would have undergone autohydrolysis, when heated at higher temperatures for prolonged periods, resulting in the precipitation of the protein-rich fraction (Anderson 1986). The drop in consistency index of the sauce by the addition of sodium alginate was 48.85 % at 0.5% concentration and 56.3% at 1.5% concentration, whereas for guar gum, it was 44.6% at 0.5% and only 14.3% at 70°C.

Serum loss and flow value : Guar gum was able to bind water so strongly that there was no serum loss during centrifugation even at the lowest concentration of 0.5%. Gum acacia gave the highest serum loss (Table 6). The serum loss decreased with increasing concentrations of the hydrocolloids in all cases. The flow value of the sauce was least, when guar gum was added. The flow value decreased with increasing concentration of hydrocolloid, indicating an increase in viscosity and thus a higher resistance to flow (Fig. 4). The flow value dropped by 63.8%, 41.8% and 35.2%, when the concentration of guar gum, sodium alginate and gum acacia in the sauce increased from 0 to 1.5%. The relationship between the flow

TABLE 6. EFFECT OF HYDROCOLLOID ON THE % SERUM LOSS IN TAMARIND SAUCE

Hydrocolloid	% conc	% serum loss
Guar gum	0.00	52.2
	0.50	0
	0.75	0
	1.00	0
	1.25	0
	1.50	0
Sodium alginate	0.00	52.20
	0.50	43.00
	0.75	38.43
	1.00	35.90
	1.25	33.11
	1.50	31.46
Gum acacia	0.00	52.20
	0.50	46.70
	0.75	42.43
	1.00	39.76
	1.25	36.59
	1.50	35.40

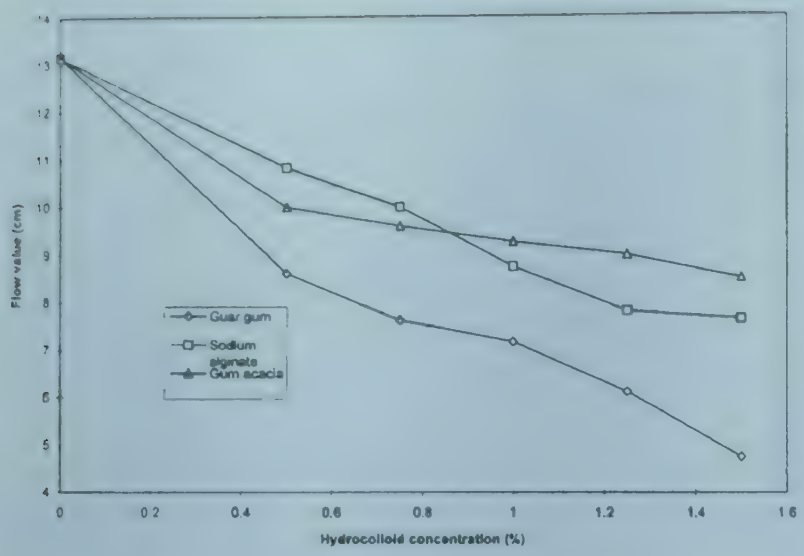

Fig. 4. Decrease in flow value of tamarind sauce with increasing hydrocolloid concentration

value and hydrocolloid concentration can be described by the following equations.

Guar gum $f = 2.219\,x^2 - 8.509\,x + 12.916$ ..... (3)

Sodium alginate $f = 0.626\,x^2 - 4.796\,x + 13.191$ ..... (4)

Gum acacia $f = 2.312\,x^2 - 6.291\,x + 12.998$ ..... (5)

where f = flow value (cm) and x = hydrocolloid concentration in percent. The r^2 values for equations 3, 4 and 5 were 0.975, 0.973 and 0.971.

The relationship between the percent serum loss and hydrocolloid concentration is described by the following equations.

Guar gum $s = 0$ .....(6)

Sodium alginate $s = 5.310\,x^2 - 21.874\,x + 52.267$ .....(7)

Gum acacia $s = 1.695\,x^2 - 14.289\,x + 52.498$ .....(8)

where s = the percent serum loss and x = hydrocolloid concentration in percent. The r^2 values for equations 6, 7 and 8 were 1, 0.998 and 0.991. Analysis of variance of the data in Table 6 was carried out to determine, if the type and concentration of hydrocolloid had a significant effect on the flow values and % serum loss (Table 7). The type of hydrocolloid had a significant effect on the flow value and % serum loss at 1% level of significance, whereas the hydrocolloid concentration significantly affected the flow value and % serum loss at 5% level of significance.

The study has clearly demonstrated the pseudoplastic behaviour of tamarind sauce. Hydrocolloid concentration had the most pronounced effect on the rheology of the sauce. The consistency of tamarind sauce can be increased by the addition of hydrocolloids with guar gum, providing maximum consistency, least serum loss and flow value, followed by sodium alginate and gum acacia. The data can be useful in process design and quality evaluation.

TABLE 7. ANALYSIS OF VARIANCE OF DATA OF FLOW VALUES

Source of variation	Degree of freedom	Sum of squares	Mean sum of squares	F-ratio
Hydrocolloid concentration	5	45.709	9.141	4.069
Hydrocolloid type	3	330.487	110.162	49.035
Error	15	33.699	2.246	-
Total	23	409.895	-	-
Analysis of variance of data of % serum loss				
Hydrocolloid concentration	5	1377.98	275.59	2.974
Hydrocolloid type	3	7919.123	2639.708	28.489
Error	15	1389.85	92.657	-
Total	23	1068.96	-	-

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Functional Properties of Defatted Cashew Kernel Flour

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Functional properties of defatted cashew (*Anacardium occidentale*) flour were compared with those of almond (*Prunus amygdalus*) and soybean (*Glycine max*). Defatted cashew kernel flour had lower water absorption capacity, higher oil absorption capacity and emulsification capacity compared to defatted soybean flour ('JS 335'). Foaming capacity of defatted cashew kernel flour was comparable with that of soy bean. Defatted kernel flour of almond exhibited lower water absorption, oil absorption, foaming capacity compared to cashew and soybean. Blending of cashew and soybean in the ratio of 1:1 improved the water absorption and emulsification capacity of cashew. Both almond and soy bean exhibited similar behaviour with respect to stability of the foam at pHs, ranging between 3 and 10. However, in the case of defatted cashew flour, foam produced was stable at pHs ranging between 3 and 9. At pHs 2 and 10, foam was unstable. Sodium chloride at concentrations ranging from 0 to 0.6 M did not affect the foam capacity, though it enhanced the extractability of the proteins from defatted flours.

Keywords : Almond, Cashew, Functional properties, Water absorption, Oil absorption, Foaming capacity, Emulsification capacity, Least gelation concentration, Soybean.

Seeds, nuts, fruits etc. are the main produce of a plant containing a large number of nutrients. These nutrients interact with each other during product development and they play an important role in determining the final quality of the product. Proteins, fats and carbohydrate are the major constituents of nuts and these constituents play an important role in product development. In this regard, a study of functional properties of defatted meal assumes importance. All plant proteins do not possess desirable functional properties. These properties could be improved by blending with other vegetable proteins or milk proteins.

Cashew (*Anacardium occidentale* L.) is one of the important horticulture crops grown in India. India has earned a total of over Rs. 2500 crore as foreign exchange by exporting cashew kernels during 1999-2000. Processing of cashew results in kernels of different grades such as wholes, splits, bits etc. While cashew wholes fetch premium price, kernels of lower grade such as bits, splits etc. fetch less. Cashew kernels from different varieties have been characterised for various biochemical constituents (Nagaraja and Krishnan Nampoothiri 1986; Nagaraja 1987 a, b; Nagaraja 1989). Although extensive data are available on the composition of cashew kernels, reports on the functional properties of defatted kernel meal are not available in literature. In order to develop value-added products from lower grade cashew kernels, it is necessary to study the functional properties of the defatted cashew kernel flour. Functional properties of proteins and isolates from winged bean (Sathe et al. 1982 a), lupin seed (Sathe et al. 1982 b), sunflower (Sosulski and Flemming 1977), alfalfa leaf protein (Wang and Kinsella, 1976), rapeseed (Amita Maheja and Saroj Dua 1995), African yam bean (Nwanekezi 1994) and peanut (Monteiro and Prakash 1994) have been studied. The present investigation deals with the functional properties of defatted cashew kernel flour and its comparison with almond (*Prunus amygdalus* L.) and soybean (*Glycine max* L.) with an objective of making suitable blended flours for developing products.

Materials and Methods

Defatted kernel flour from cashew kernel of different grades procured from M/s Achal Industries was prepared by extracting with n-hexane at ambient temperature (25-30°C) over a period of 48 h. Similarly, almond kernels of widely cultivated variety procured from Central Institute of Temperate Horticulture, Srinagar and commercially purchased were soaked in distilled water for 15 min. and testa peeled off. Kernels were dried at 70°C for 6 h and then defatted. Soybeans ('JS 335') obtained from National Research Centre for soy bean Indore, were soaked in distilled water for 1 h and outer skin was removed and dried at 70°C for 6 h. Dried beans were defatted and powdered. Defatted flour was sieved through 60 mesh and used for analysis. Double refined groundnut oil, sunflower oil, coconut oil and palmolein oil were purchased from local market.

Functional properties of defatted flour viz., water and oil absorption capacity (Sosulski 1962), least gelation concentration and foaming capacity (Coffmann and Garcia 1977) and emulsification capacity (Beuchat 1977) were data determined. Defatted kernel flour was analysed for proteins (AOAC 1980), sugars (Dubois et al. 1951) and carbohydrates (Clegg 1956). The conversion factor of 6.25 was used for calculating the protein content of different defatted flours. Defatted flour was extracted with hot 80 % ethanol to remove free sugars, amino acids and phenols and the residue was dried at 70°C for 2 h. The dried residue was digested at ambient temperature (27 to 30°C) with 52% perchloric acid for 15 min and the digest was made upto a known volume. Liberated sugars were estimated and starch value was computed using a conversion factor of 0.9. For extractability studies, defatted flour was stirred with distilled water (1:20 w/v) at different pH/sodium chloride at different concentrations for 60 min at ambient temperature and centrifuged. Supernatant was used for protein estimation.

Results and Discussion

Understanding of the functional properties of any defatted meal is essential for the assessing the potential of proteins in the food system (Kinsella 1976). Functional properties like water absorption capacity, oil absorption capacity, foam capacity, emulsification capacity and least gelation concentration of any defatted meal determine its utility in developing food formulations. Defatted flour of cashew, almond and soybean and their blends were analysed for sugars, starch and proteins (Table 1). In the present investigation, values obtained for various biochemical constituents of almond are comparable with those reported by Kumar and Uppal (1995) for different selections of almond from Jammu and Kashmir. Further, wide variations with respect to composition have been noticed by the same authors. Composite flour mix of cashew and soybean had lower sugar, starch and protein contents compared to cashew.

TABLE 1. BIOCHEMICAL COMPOSITION OF DEFATTED KERNEL FLOUR

Defatted flour	Sugars, %	Carbohydrates, %	Proteins, %
Cashew			
Whole kernels	12.9	20.3	63.5
Small dessert pieces	13.1	21.8	55.3
Baby bits	17.3	15.8	56.1
Almond			
Commercial sample	7.9	14.0	55.3
CITH sample	5.1	10.2	52.2
Soybean			
'JSS 335'	8.3	12.8	52.2
Composite flour			
Cashew (whole kernel) : Soybean ('JS 335')	8.4	14.8	45.5
Cashew (whole kernel : Almond ('CITH') (1:1)	8.2	13.9	40.8
Almond ('CITH' : Soybean ('JSS 335') (1:1)	5.1	7.8	52.3
Cashew (whole kernels) : Almond ('CITH' : Soybean ('JSS 335') 1:1:1)	6.3	7.5	50.8
CD (1%)	3.77	5.39	8.55

Values are mean of 3 individual estimations

Functional properties of defatted cashew kernel flour prepared from different grades of processed cashew kernel, almond and soybean have been studied and the results are presented in Table 2. Water absorption capacities of defatted kernel flours obtained from different grades of cashew and almond were less compared to soybean. Water absorption capacity of soy bean observed in the present investigation was less compared to the reported value (Srinivas and Narsinga Rao 1986; Sharma and Subramanian 1994). This could be due to varietal variations. Lower water absorption values of defatted flour of cashew kernel and almond indicate that the defatted flours of these are less hydrophilic in nature compared to soybean. As polar amino acids are the ones, which are responsible for binding of water molecules by proteins, differences in water absorption capacity values might suggest the variations in the polar amino acid composition cashew, almond and soybean. Blending of defatted cashew kernel flour with either almond or soybean resulted in higher water absorption capacity, thereby suggesting that defatted cashew kernel flour and soybean in the ratio of 1:1 could be useful in developing bakery products for which hydration property for improvement of handling characters is required.

Oil absorption capacity of defatted cashew kernel flour (128.8 %) was more than that of almond (115.5 %) and soybean (96.8 %). Blending of defatted cashew kernel flour with either almond or soybean did not improve the oil absorption capacity of cashew kernel flour. The values obtained for oil absorption capacity of soybean were less, compared to those reported in literature (Srinivas and Narasinga Rao 1986; Sharma and Subramanian 1994). This could be due to varietal variations. Higher oil absorption capacity exhibited by defatted cashew kernel flour compared to either almond or soybean indicated the higher level of non-polar amino acids in cashew kernel

TABLE 2. FUNCTIONAL PROPERTIES OF DIFFERENT DEFATTED FLOURS

Defatted flour	Water absorption capacity, %	Oil absorption capacity, %	Least gelation conc, %	Foaming capacity, (% volume increase)	Emulsification ml oil/g flour	capacity, ml oil/g protein
Whole kernels	56.3	128.8	6.0	53.0	33.7	51.1
Cashew kernel - Baby bits	59.8	100.4	9.0	10.0	32.5	58.6
Cashew kernel - Small dessert pieces	48.8	84.0	7.5	8.0	30.8	55.8
Almond kernel - Commercial sample	36.7	84.4	10.0	20.0	27.4	48.9
Almond kernel - 'CITH' sample	46.8	115.5	6.0	42.0	32.5	63.0
Soybean 'JS 335'	100.2	96.8	15.0	50.0	23.0	34.7
Cashew (whole kernel) : Soybean ('JS 355') (1:1)	73.7	119.3	9.0	37.0	64.4	140.4
Cashew (whole kernel) : Almond ('CITH') (1:1)	114.3	138.5	9.0	40.0	68.9	168.6
Almond ('CITH') : Soybean ('JS 335') (1:1)	86.2	117.1	12.0	38.0	63.6	121.1
Cashew (whole kernel) : Almond: Soybean (1:1:1)	81.1	124.7	10.0	28.0	67.6	132.5
CD (1%)	13.87	17.01	-	10.09	7.41	19.13

Values are mean of 3 individual estimations

protein compared to almond and soybean. Higher carbohydrate content (starch) in the defatted cashew kernel flour (Table 1) compared to either almond or soybean could also contribute to the higher oil absorption by defatted cashew kernel flour.

Results presented in Table 2 indicate that the defatted cashew kernel flour prepared from whole cashew kernels had better gelling properties with least gelation concentration of 6 % compared to either almond or soybean. Blending of cashew with either almond or soybean in the ratio of 1:1 had negative effect, as the least gelation concentration of composite flour mix increased from 9 to 10 %.

Foaming capacity of defatted flour of whole cashew kernel (53 %) was higher compared to almond (42 %) and soybean (50 %). Foaming capacity of cashew kernel flour was reduced, when it was blended either with almond (40 %) or soybean (28 %). Thus, blending of defatted cashew kernel flour with either almond or soybean did not improve the foaming capacity. Foaming capacity of soybean (50 %) obtained in the present investigation was less compared to the reported value of 132 % at pH 5.0 (Srinivas and Narasinga Rao 1986; Sharma and Subramanian 1994). Lower foaming capacity could be due to varietal variations.

Emulsification capacities of different defatted flours prepared from whole cashew kernel, small dessert pieces (SDP) and baby bits (BB) ranged from 30 to 32. Defatted soybean flour exhibited lower emulsification capacity (22.7 %). This is in agreement with the reported value of around 25 at pH 5 (Srinivas and Narasinga Rao 1986). Emulsification capacity of cashew kernel flour could be improved by blending with either almond or soybean in the ratio of 1:1. Improvement of defatted cashew flour by blending with particularly soybean with respect to emulsification capacity could help in the preparation of milk from composite flour mix.

Absorption of different vegetable oils by defatted cashew, almond and soybean flours and their blends was studied and the results are presented in Table 3. Absorption of refined groundnut oil, sunflower oil, coconut oil and palmolein oil by defatted cashew kernel flour was higher compared to soybean. This could be due to higher starch content in defatted cashew kernel flour (20.3 %) compared to soybean (12.8 %) (Table 1). Blending of cashew flour with soybean flour reduced the oil absorption capacity. Composite flour mix of cashew and soybean in the ratio of 1:1 had lower starch content (14.8 %) compared to cashew. Blending of cashew with almond slightly improved the oil absorption. As the starch content of composite flour mix of cashew and almond (13.9 %) was still less than that of cashew (20.3 %), increased oil absorption by composite flour mix could not be attributed to starch content. Higher oil absorption by composite flour mix of cashew and almond could be due to other reasons, which need investigation.

Stability of the foam of defatted kernel flour of cashew (whole kernels), almond and soybean as a function of pH ranging from 2 to 10 was studied (Fig. 1 to 3). At extreme pHs of 2 and 10, foam produced by defatted cashew kernel flour was very unstable. Between pHs 3 and 9, foam produced by defatted cashew kernel flour was quite stable. In the case

TABLE 3. OIL ABSORPTION CAPACITY OF DIFFERENT DEFATTED KERNEL FLOURS

Defatted kernel flour	Refined sunflower oil, %	Coconut oil, %	Palmolein oil, %
Whole cahew kernel	120.5	126.5	99.3
Cashew kernel - Baby bits	79.9	87.9	96.5
Cashew kernel - Small dessert pieces	76.8	81.2	85.9
Almond kernel - Commercial sample	137.4	139.7	110.2
Almond kernel - 'CITH' sample	152.4	155.2	120.5
Soybean 'JS 335'	91.9	96.9	88.6
Cashew (whole kernel) : Soybean ('JS 335') (1:1)	108.9	109.2	103.2
Cashew (whole kernel) : Almond ('CITH') (1:1)	124.4	149.7	126.8
Almond ('CITH') : Soybean ('JS 335') (1:1)	117.9	137.4	117.1
Cashew (whole kernel) : Almond : Soybean (1:1:1)	111.9	122.2	108.4
CD (1%)	10.01	6.93	9.92

Values are mean of 3 individual estimations

of almond, foam stability decreased with increase in pH from 3 to 10. Foam produced at pH 2.0 was quite stable. In the case of soybean also, foam produced at pHs ranging from 3 to 10 decreased. At pH 2, defatted soybean flour produced stable foam. However, minimum foaming capacity and stability at pH 4 has been reported for soybean meal (Srinivas and Narasinga Rao 1984; Sharma and Subramanian 1994). Compared to almond and soybean, foam stability of cashew at pHs ranging from 3 to 9 was better. This could reflect on the solubility properties of defatted flour. Products could be developed from defatted cashew flour over a wide range of pH from 3 to 9. This could be an added advantage, while developing products for which foaming is required.

Extractability of proteins from defatted flour as a function of pH was studied and the results are depicted in Fig. 4. Extractability of protein increased with pH in the case of almond ('CITH'), cashew kernel baby bits (BB) and small desert pieces (SDP). In the case of defatted kernel flour of cashew whole kernels, soybean and almond (commercial sample) extractability at pH 2 and 10 was higher. Extractability of proteins between pH 3 and 9 did not vary much. Between pHs 3 and 9, a gradual increase in the extractability of proteins was observed. Differences observed in the foaming capacity and its stability at different pHs could be due to differences observed in the extractability proteins.

Extractability of proteins from defatted flour at different sodium chloride concentrations (0 to 0.6 M) was studied and the results are given in Fig. 5. Gradual increase in the extractability of protein was noticed. Although sodium chloride at different concentrations enhanced the extractability of proteins, it did not influence the foaming capacity of the defatted kernel flour in the present investigation. Sodium

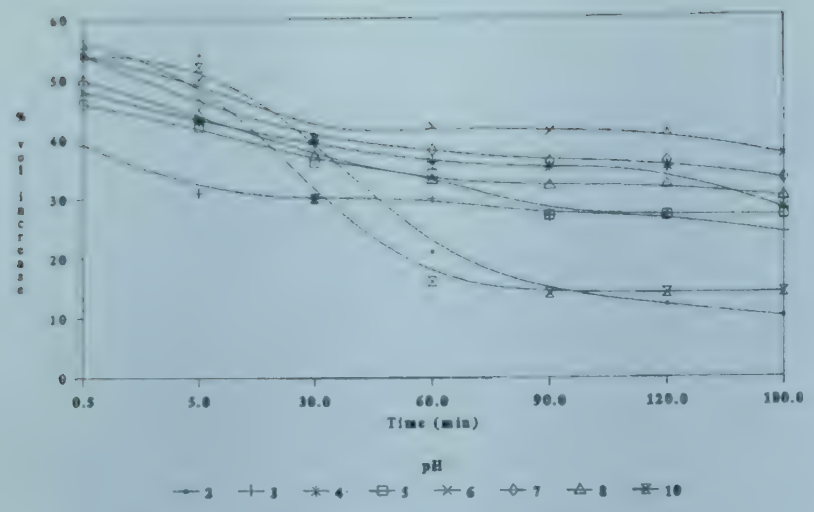

Fig. 1. Effect of pH on foam stability of defatted kernel flour from whole cashew kernels

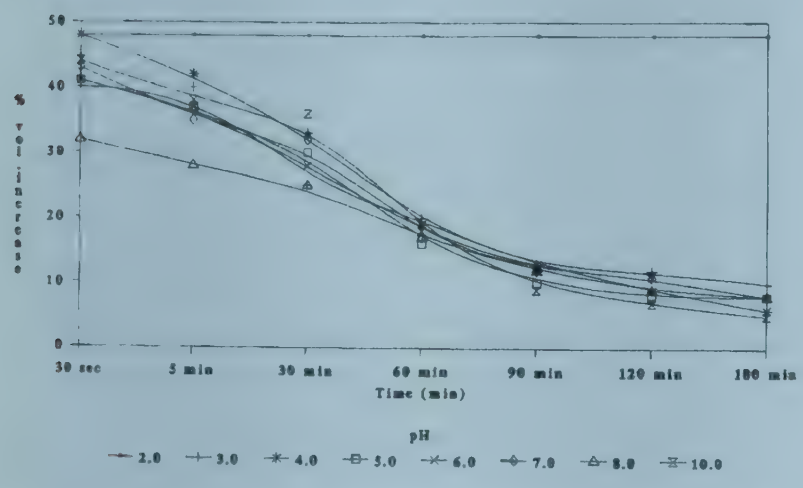

Fig. 2. Effect of pH on foam stability of defatted almond kernel flour ('CITH' sample)

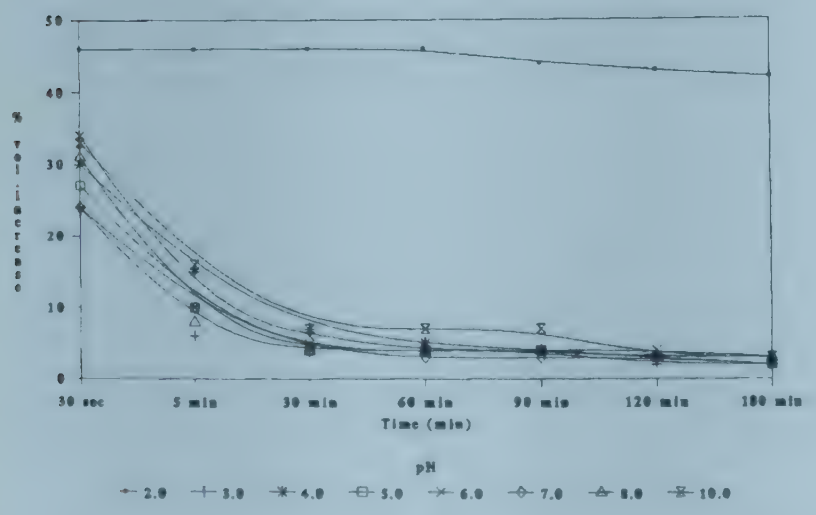

Fig. 3. Effect of pH on foam stability of soybean defatted flour ('JS 335')

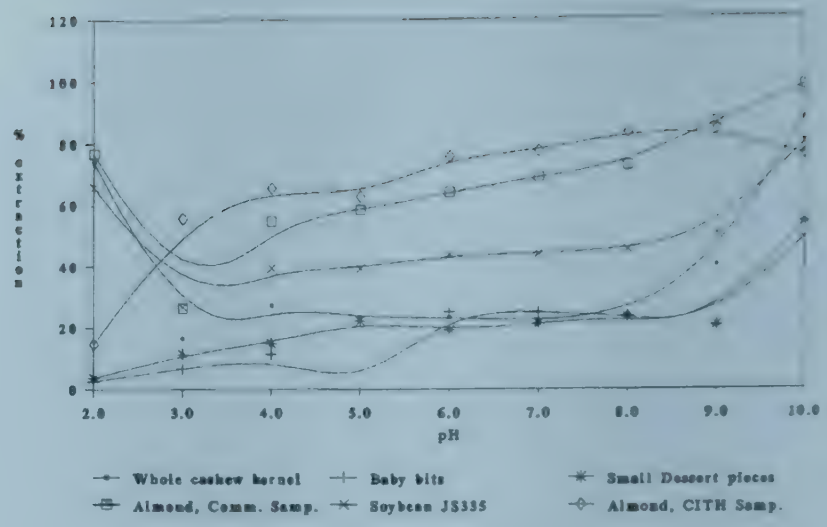

Fig. 4. Effect of pH on protein extractability

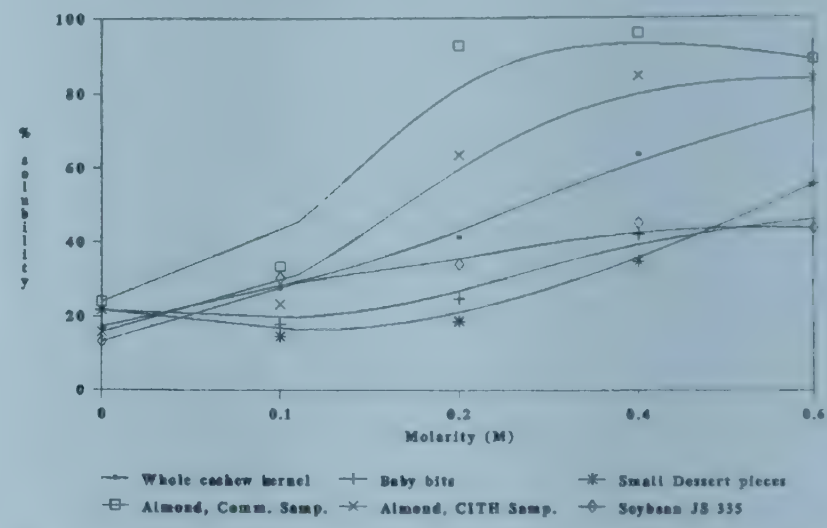

Fig. 5. Effect of NaCl concentration (%) on protein extractability

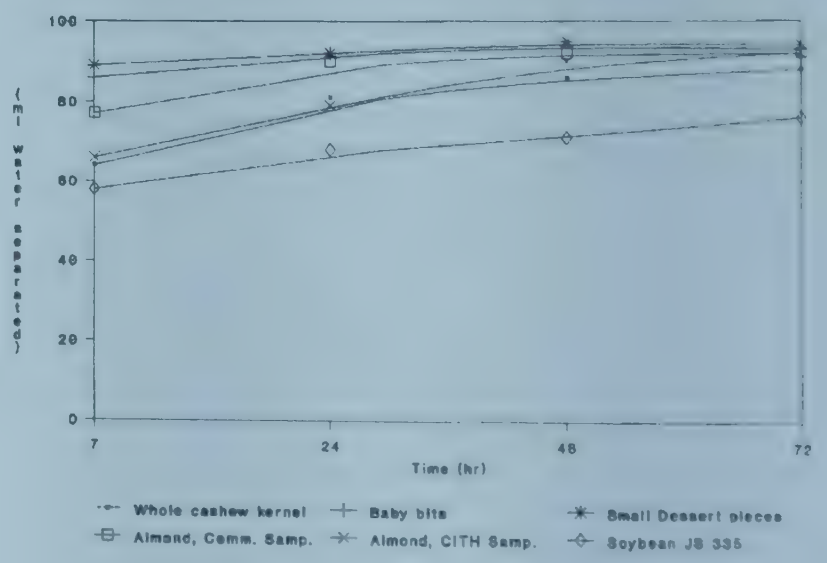

Fig. 6. Emulsion stability

chloride at concentrations ranging from 0.2 to 1 M has been shown to influence foaming capacity of soybean meal (Sharma and Subramanian 1994).

Stability of the emulsion formed with double refined groundnut oil and different defatted kernel flours was studied and the results are presented in Fig. 6. None of the emulsions formed was stable and considerable quantity of water got separated within few minutes after emulsion formation. Between 7 h and 72 h, quantity of water further separated was less. Results have indicated that stable emulsion could not be produced from defatted cashew kernel flour and it may require addition of stabilisers for getting stable emulsions.

Conclusion

Results obtained in the present study have clearly indicated that functional properties of defatted cashew kernel

flour could be improved by blending with soybean for its water absorption capacity and emulsification capacity. Defatted cashew kernel flour in combination with soybean could be used in developing bakery products, where hydration properties for improvement of handling characters are required. Further, cashew offers additional advantage of producing stable foam over a wide pH range from 3 to 9. With its low least gelation concentration of 6 %, it could be used in developing products, where gelling is required.

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RESEARCH PAPER

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Comparative Analysis of Crocetin and Its Glycosyl Esters from *Crocus sativus* L. and *Crocus haussknechtii* Boiss. as An Alternative Source of Saffron

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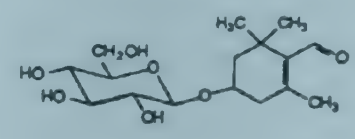
Stigmata of *Crocus sativus* L. and *Crocus haussknechtii* Boiss. were extracted and analyzed by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). Their carotenoid composition showed strong similarity among all the crocetin-glycosyl esters, present in them, with minor quantitative differences of more polar components. Crocetin aglycons were also isolated from stigmata samples and characterized by analytical TLC and HPLC, ultraviolet-visible (UV-vis) and high resolution Nuclear Magnetic Resonance (^1H and ^{13}C NMR) spectroscopy. The relatively high concentrations of pigments in *C.haussknechtii* stigmata and the similarity of carotenoid composition of this species with those of *C. sativus* showed that some of the wild species of *Crocus* can be used as a potential source of saffron.

Keywords : *Crocus sativus* L., *Crocus haussknechtii,* Crocetin glycosyl esters, UV-vis spectroscopy, NMR spectroscopy, Saffron.

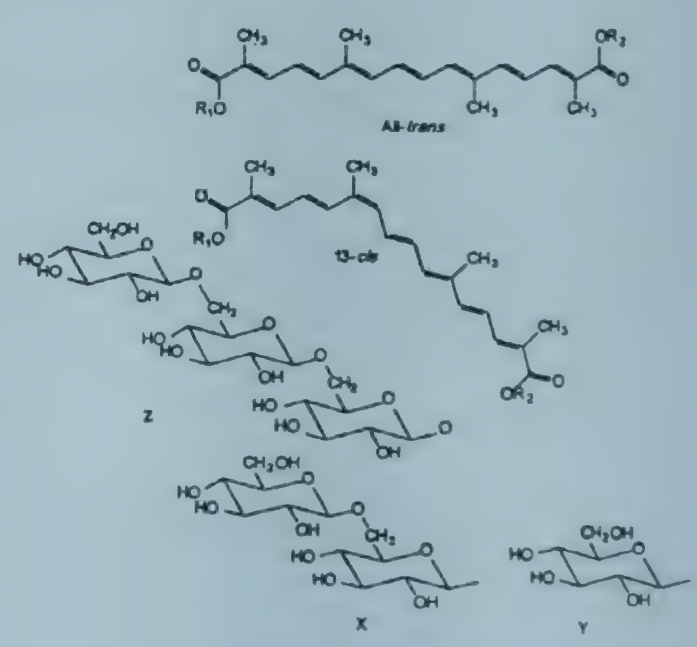
Crocus species are herbaceous, perennial and mostly ornamental plants from Iridaceae family. The saffron crocus (*Crocus sativus* L.) was the first crocus to be cultivated by man and found to be an economic plant grown in various parts of the world such as Iran, India, China, Spain, Greece and Morocco. The utilized part, commonly known as saffron, is the dry stigmata. Saffron has a wide variety of applications in food industries as a flavouring and colouring agent and it has also been used in perfumery and as a drug in ancient medicine (Mathew 1982; Assimiadis et al. 1998; Corti et al. 1996). The most important secondary metabolites in saffron are glycosyl esters of crocetin, highly water soluble carotenoids and picrocrocin. Picrocrocin is a monoterpene glycoside, which is responsible for the bitter taste of saffron and is the precursor of the odour - active safranal.

The colouring components of saffron are *cis* and *trans*-crocins (CRCs), which are unusual water soluble carotenoids. In fact, crocins are glycosyl derivatives of the C_{20}-dicarboxylic acid crocetin (8,8'-diapocarotene-8,8'-dicarboxylic acid) (CRT) (Fig. 1). The structural study of these compounds has been performed by many groups (Karrer and Miki 1929; Kuhn and L'Orsa 1931; Pfander and Wittwer 1975 a,b; Pfander and Rychener 1982; Tarantilis et al. 1994, 1995; Pfister et al. 1996; Corti et al. 1996; Van Calsteren et al. 1997; Assismiadis et al. 1998 and so far, seven different glucosyl esters of crocetin have been isolated and characterized from saffron and garden crocuses (Fig. 1). Among these compounds Crocin-5, the digentiobiosyl ester of crocetin, is the principal pigment in saffron.

C. haussknechtii (Boiss. & Reuter ex. Maw) Boiss. is one of the wild species of *Crocus* in Iran that grows in dry fields or rocky hillsides, or in sparse oak scrub of western and southern areas of Iran (Hamedan, Kermanshah and Fars provinces). *C. haussknechtii* is a autumn-flowering species and



Picrocrocin $C_{16}H_{26}O_7$



Crocins (CRCs): Glucosyl esters of crocetin

Crocin-7: R_1 = R_2 = Three β-D-glucosyl (Z)	$C_{84}H_{134}O_{54}$
Crocin-6: R_1 = Three β-D-glucosyl (Z), R_2 = β-D-gentiobiosyl (X)	$C_{80}H_{124}O_{49}$
Crocin-5: R_1 = R_2 = β-D-gentiobiosyl (X)	$C_{44}H_{64}O_{24}$
Crocin-4: R_1 = β-D-gentiobiosyl (X), R_2 = β-D-glucosyl (Y)	$C_{38}H_{54}O_{19}$
Crocin-3: R_1 = β-D-gentiobiosyl (X), R_2 = H	$C_{32}H_{44}O_{14}$
Crocin-2: R_1 = R_2 = β-D- glucosyl (Y)	$C_{32}H_{44}O_{14}$
Crocin-1: R_1 = β-D- glucosyl (Y), R_2 = H	$C_{26}H_{34}O_9$
Crocetin (CRT):R_1 = R_2 = H	$C_{20}H_{24}O_4$

Fig. 1. Chemical structures of the crocetin derivatives and picrocrocin

* *Corresponding Author*

has a red and three-branched stigma as *C.sativus*. In taxonomic classifications of *Crocus* genus, *C. sativus* and *C. haussknechtii* are grouped in *Crocus* section and *Crocus* series (Mathew 1982). Comparative studies of the corm tissue proteins of Iranian *Crocus* species have been carried out and the results have shown that *C. haussknechtii* is very similar to *C. sativus* (Ebrahimzadeh et al. 1998).

The objective of this study was to focus on qualitative and quantitative comparison of carotenoid pigments from stigmata of *C. sativus* and *C. haussknechtii,* a wild species of *Crocus* by chromatographic and spectroscopic techniques (TLC, HPLC, UV-vis and NMR spectroscopies).

Materials and Methods

Plant materials : Flowers of *C. sativus* were collected from the saffron field at Research Station of Tehran University and flowers of *C. haussknechtii* were harvested from their natural habitat at the rocky hillsides of Fars province of Iran during the second half of November 1998. The stigamta were separated, air dried at 25°C in the dark and stored at 4°C, until further analysis.

Extraction of the dried stigmata : For extraction of carotenoid components, dried samples of stigmata were ground, suspended in 50% methanol (5 mg/ml) and stirred for 1 h at room temperature in darkness. Later, the suspensions were centrifuged at 30000 x g and at 10°C for 20 min to separate the vegetable tissue residue. Finally, the supernatants were filtered through a 0.45-µm acetate cellulose filter (Sartorius GmbH, Germany) and stored at –20°C, until analysis.

Isolation of crocetin aglycons from stigmata : Dried stigmata (5 mg) were successively and exhaustively extracted with light petroleum (b.p. 40-60°C), diethyl ether (0.03% BHT) and methanol in a Soxhlet extractor in the dark. The methanolic extracts containing glucoside carotenoids (CRCs) were evaporated to dryness in the dark at 25°C using a Rota Vapor (Heidolph, Germany). Crocetin aglycons were obtained from dried methanolic extracts of samples in basic (1% KOH) and acidic (1% HCl) solutions. The precipitated crocetins were washed three times with distilled water to free acid, lyophilized for 24-36 h and stored at –20°C. For quantitative analyses, the lyophilized aglycons were dissolved in dimethyl sulfoxide (DMSO) and passed through a 0.45-µm filter.

Standards : Authentic crocetin was obtained as the pyridine salt from Sigma (St. Louis, MO, USA) (Cat. No. C 3398). The crystals were washed with CH_3OH/C_6H_6(4:1) to remove pyridine prior to chemical analysis.

An authentic sample of saffron (a crude source of crocin) was obtained from Sigma (St. Louis, MO, USA) (Cat. No. S 8381) and used for comparison and quantitative analysis of carotenoids in samples.

Quantitative analysis of crocin and picrocrocin : Powdered samples of stigmata (2 mg) were extracted with distilled water (5 ml) in 20-ml ground-glass stoppered Pyrex test tubes in a boiling water bath for 20 min, with occasional shaking to ensure that the stogmata are properly immersed in the distilled water. The extracts were cooled to room temperature and the absorbance of the supernatant solutions was determined against a water blank at 440 nm. Determinations were performed in triplicates (Basker and Negbi 1985). Crocin and picrocrocin contents of samples were quantified through their molar absorptivities in water : ε_{440} = 133750 $M^{-1}cm^{-1}$ (Corradi and Micheli 1979) and ε_{250} = 10100 $M^{-1}cm^{-1}$ (Himeno and Sano 1987), respectively. The results were subjected to statistical analysis at 5% and 1% levels, using Duncan's Multiple Range Test (DMRT).

UV-visible spectroscopy : UV-visible spectra of DMSO solutions of authentic crocetin and purified crocetins were obtained, using a Shimadzu (Tokyo, Japan) UV-160 A, UV-vis recording spectrophotometer. The scanning range was from 200-550 nm. The spectra of aqueous stigmata solutions were also obtained between 200-500 nm.

Thin layer chromatography : DMSO solutions of purified crocetins, authentic crocetin and methanolic extracts of dried stigmata were used for analysis by TLC, using n-butanol : acetic acid : water (4:1:1 v/v) as the mobile phase. The stationary phase was silica gel G60 (Merck), the thickness was 0.3 mm, on 20x20 standard sized plates. A 5-µL aliquot of the each DMSO solutions of purified crocetins, a 100-µL aliquot of the each alcoholic solutions of crude extracts together with a 1-µL aliquot of the DMSO solution of standard crocetin were spotted onto a TLC plate.

High performance liquid chromatography : Crocetin aglycons and crocins were identified by analytical HPLC, using a Shimadzu LC-6A liquid chromatograph, equipped with a 300 mm length x 3.9 mm ID µ-Bondapak C_{18} (10 µm particle size) column from Waters, and a SPD-6AV UV-visible spectrophotometric detector. The mobile phase was an isocratic solvent system, consisting of 1% (v/v) acetic acid in methanol and 1% (v/v) acetic acid in water (60:40) with an elution profile from 0-40 min at a flow rate of 0.5 ml/min and sample saizes 10 µl of the each crude methanolic extracts (1 mg/ml) for the separation of crocins and up to 60 min at a flow rate of 1 ml/min and sample sizes 5 µl of the each DMSO solutions (0.2 mg/ml) for the identification of crocetins. Detection was performed at 440 nm.

Lichrosolv (Merck) solvents were used for the chromatography and all solvents were filtered through a 0.45-µm filter before use.

NMR spectroscopy : ^1H and ^{13}C NMR spectra of purified crocetins were recorded at 25°C in DMSO-d_6 on a Bruker DRX-500 Avance spectrometer (500.13 MHz for ^1H and 125.77 MHz for ^{13}C) equipped with a 5-mm BBi (Broad Band inverse) probe. ^1H and ^{13}C NMR spectra of DMSO were at 2.49 and 39.5 ppm, respectively. Connectivities between protons were obtained using ^1H ^1H 2D COSY.

Results and Discussion

Quantitative analysis of crocin and picrocrocin : The results obtained from quantitative analysis of carotenoids (values are expressed as crocin) and picrocrocin content of stigmata samples are shown in Table 1.

Based on the ratio that existed between crocin and crocetin

TABLE 1. CROCIN, CROCETIN AND PICROCROCIN CONTENT OF STIGMATA SAMPLES

Sample	Crocin, mg/100 mg DW	Crocetin, mg/100 mg DW	Picrocrocin, mg/100 mg DW
C. sativus stigma	$15.26^{a} \pm 0.61$	$5.19^{a} \pm 0.21$	$11.62^{a} \pm 0.91$
C. haussknechtii stigma	$8.42^{b} \pm 0.32$	$2.86^{b} \pm 0.11$	$7.37^{c} \pm 0.49$
standard saffron	$4.91^{c} \pm 0.39$	$1.67^{c} \pm 0.13$	$9.18^{b} \pm 0.75$

Means in columns that are not followed by the same letters are significantly different at the 1% and 5% levels using DMRT

values (Basker and Negbi 1985), carotenoids content of stigmata samples could also be expressed as crocetin percent (Table 1).

The carotenoids and picrocrocin concentrations of the *C. haussknechtii* stigmata were compared with the standard saffron and saffron obtained from cultivated *C. sativus*. Although the concentration of crocin in *C. haussknechtii* stigmata was lower than the cultivated saffron, it was 1.7 times higher than that of the authentic saffron.

UV-visible spectra : The UV-visible spectra of DMSO aglycons solutions showed characteristic spectra of the carotenoid moiety in the molecule, with double peaks in the visible region (Fig. 2). The absorption band between 400 and 500 nm with λ_{max} at 434.5 mm can be characteristic of all *trans*-carotenoids and is sensitive to solvents (Tarantilis et al. 1994).

The spectra of DMSO solutions of isolated aglycons and standard crocetin were very similar. The absence of a peak between 320 and 340 nm in the isolated aglycons spectra, which were characteristics of the 13-*cis*-carotenoids (Tarantilis et al. 1994, 1995) and identical to standard crocetin suggested that isolated aglycons were in the form of *trans* isomer.

The UV-visible spectra of aqueous stigmata solutions had an absorption band between 400 and 500 nm, with λ_{max} at 440 nm. The quantitative analysis of carotenoids was performed at 440 nm.

Thin layer chromatography : Six yellow pigments were detected at R_f values of 0.29, 0.43, 0.56, 0.63, 0.80 and 0.96 on the TLC plate of crude methanolic extracts. When, higher concentrations of extracts were analyzed, a new weak yellow spot also appeared at R_f value of 0.19 in both TLC chromatograms. Only one yellow spot at R_f value of 0.96 was

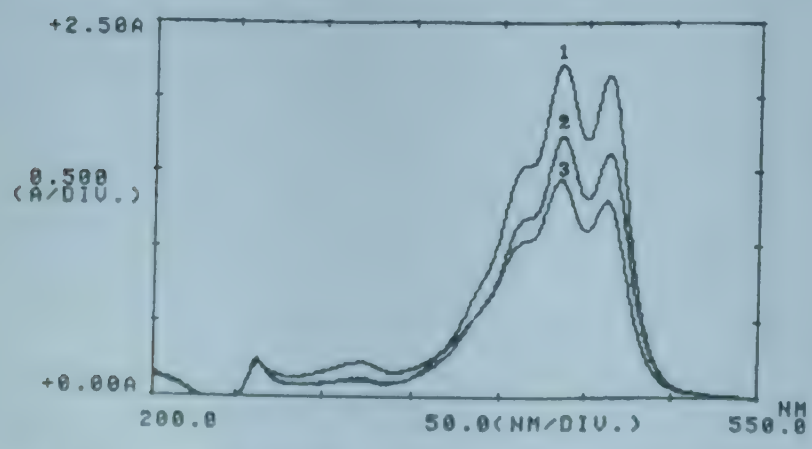


Fig. 2. UV-visible spectra of DMSO solutions of standard crocetin (1) and isolated crocetins from *C. sativus* (2) and *C. haussknechtii* (3) stigmata

observed in TLC chromatograms of isolated aglycons and standard crocetin.

A comparison between R_f values of spots corresponding to isolated aglycons and last spot in TLC chromatograms of crude extracts with R_f value of standard crocetin confirmed the purity of isolated aglycons and also the existence of small amounts of free crocetin in stigmata samples.

In order to identify the other isolated pigments, the spots were collected from the plate and after discarding the TLC solid support, their aqueous solutions were analyzed by UV-visible spectrometry. Crocin-5 identification (a major yellow pigment appearing at R_f value of 0.43 on TLC plate) was determined by its spectral characteristics (λ_{max} H_2O = 440 nm). The other yellow pigments, which had UV - visible spectra identical to Crocin-5 spectrum might be different glycosyl esters of crocetin (Fig. 1).

It was apparent, that two minor yellow pigments with R_f values of 0.19 and 0.29 show the existence of more polar compounds than Crocin-5, probably hexa- and pentaglycosides of crocetin (Crocin - 7 and - 6, Fig. 1) in stigmata samples.

High performance liquid chromatography : Fig. 3 a shows the chromatographic profile of a methanolic stigmata extract of *C. sativus* monitored at 440 nm. Seven principal peaks (numbers 1-7) were detected at retention times of 6.6, 7.3, 8.5, 11.3, 15.8, 23.2 and 34.5 min, respectively. Besides the peaks 3 to 7, which were already known as tetra - to monoglycosides of crocetin (Crocin - 5 to -1), the two other small peaks (numbers 1 and 2) appeared in the chromatogram. These pigments, which were more hydrophilic than Crocin-5, are identical with the hexa - and penta-glycosyl esters (Crocin - 7 and - 6) and occurred as main components in garden crocusses (Pfander and Rychener; 1982; Pfister et al. 1996). These pigments were also detected using TLC (Yellow spots with R_f values of 0.19 and 0.29).

As can be seen in HPLC chromatogram of crude extract of *C. haussknechtii* stigmata (Fig. 3 b), seven principal peaks were observed at retention times of 6.4, 7.1, 8.6, 11.2, 15.5, 23.1 and 34.3 min, respectively. Like HPLC chromatogram of *C. sativus,* each peak identified one of the glycosyl esters of crocetin. In contrast to *C.sativus* stigmata, the two more polar compounds (peaks 1 and 2) were major components in those of *C. haussknechtii*. This suggested that in wild *Crocus* species, Crocin-5 might not be essentially the only major pigment of stigmata.

The chromatograms also showed the quantitative composition of the pigments in the stigmata samples. The amount of each pigment was determined as the percentage of peak area, corresponding to it to the total area integrated for all peaks in the chromatograms. As expected, the main pigment in *C. sativus* stigmata was Crocin-5 (peak number 3, *ca.* 50%). Other crocins (peak numbers 1, 2, 4, 5, 6 and 7) included 2, 1, 28, 2, 5 and 7% of the total pigments, respectively. Different crocins (Crocin - 7 to - 1) also included 18, 15, 26, 17, 6, 4 and 8% of the total in *C. haussknechtii* stigmata, respectively.

The chromatographic profiles of DMSO solutions of standard crocetin and isolated aglycons, obtained at 440 nm.

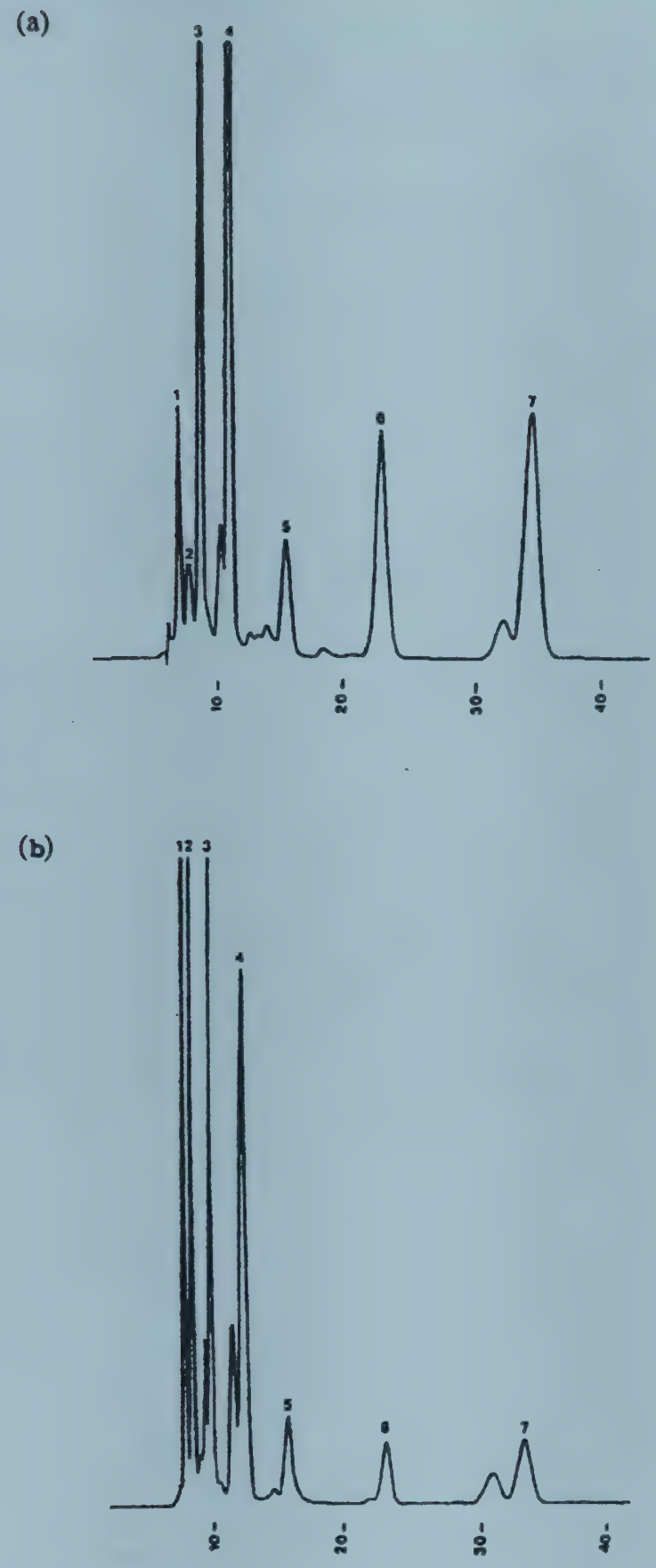


Fig. 3. HPLC chromatograms of methanolic stigmata extracts of *C. sativus* (a) and *C. haussknechtii* (b) at 440 nm

Only a single sharp peak appeared in all chromatograms and its retention time varied from 56.5 min for standard crocetin to 56.7 and 56.8 min for isolated aglycons from *C. sativus* and *C. haussknechtii* stigmata, respectively. As reported by earlier authors (Pfander and Rychener 1982; Tarantilis et al. 1994), the compounds such as crocetin with free carboxyl groups were detected at extremely long retention times.

NMR spectroscopy : Complementary results on structural specifications of isolated aglycons were obtained by NMR spectroscopy. ^{1}H and ^{13}C NMR data provided useful information for identification of the compounds that could be isolated. ^{1}H NMR spectra of isolated aglycons were obtained in DMSO-d_6 and compared with ^{1}H NMR spectrum of standard crocetin. The values of chemical shifts and coupling constants for the protons of the unsaturated chain of isolated aglycons were determined. In the ^{1}H NMR spectra of isolated aglycons (crocetin$_{c.s}$ and crocetin$_{c.h}$, isolated aglycons from *C. sativus* and *C. haussknechtii* stigmata, respectively) there was a broad doublet at 7.194 ppm for crocetin$_{c.s.}$ and at 7.195 ppm for crocetin$_{c.h}$ coming from proton 10 coupling with proton 11 ($J_{c.s} = J_{c.h} = 11.6$ Hz), a doublet of doublets at 6.600 ppm for crocetin$_{c.s}$ and 6.606 ppm for crocetin$_{c.h}$ due to proton 11, which was coupled with proton 12 ($J_{c.s} = 14.9$ Hz, $J_{c.h} = 14.8$ Hz), and also a doublet of doublets at 6.470 ppm for crocetin$_{c.s}$ for protons 14 and 14' as a result of coupling with protons 15 and 15' ($J_{c.s} = J_{c.h} = 11.6$ Hz). There was also a similar type of coupling for protons 15 and 15' at 6.819 ppm for crocetin$_{c.s}$ and at 6.820 ppm for crocetin$_{c.h}$ ($J_{c.s} = J_{c.h} = 14.4$ Hz). According to Van Calsteren et al (1997), the 14-Hz value is characteristic of a *trans* configuration for the 15-15' double bond.

A comparison between 'H NMR spectra, chemical shifts and coupling constants for the protons of olefinic region of isolated aglycons with those of standard crocetin proved the chemical nature of them.

Connectivities between protons (14/15, 11/12 and 10/11) were also obtained using 2D COSY (data are not shown here). The 2D NMR spectra of the unsaturated chain of isolated aglycons showed completely similar profiles with the pattern of a *trans* isomer form of aglycon (Van Calsteren et al. 1997; Assimiadis et al. 1998).

All the above observations have been confirmed by the ^{13}C NMR data (data are not shown here). The chemical shift values of the olefinic region cabrons of the isolated aglycons were clearly comparable with those of standard crocetin in DMSO-d_6. These data provide further evidence as to the identity of the isolated aglycons.

Conclusions

The aim of the present study was to obtain and characterize crocetin and its glycosyl esters from stigmata of *C. sativus* and *C. haussknechtii* (one of the wild species of *Crocus)* with different available methods. The results from analytical TLC and HPLC techniques showed that all of the glycosidic carotenoids, which were found in *C. sativus* stigmata were also present in *C. haussknechtii* stigmata. HPLC results also showed that this method not only would prove to be simple and efficient for separation of crocetin glycosides in samples but also the most suitable one for the identification of free crocetin in crude extracts. Development of a method for the preparative-scale assays will certainly pave the way for industrial production of free crocetin and its glycosyl esters from samples.

Spectroscopic methods could be useful to confirm the structural features of isolated crocetin aglycons from stigmata samples. The absence of an absorption peak between 320-340 nm in the UV region of UV-visible spectra of isolated aglycons, which were characteristics of most mono-*cis*-carotenoids and also ^{1}H NMR data supported that like standard

crocetin, the separated aglycons are also in all - *trans* isomer form.

Due to the similarity between carotenoid composition of *C. sativus* and that of *C. haussknechtii* stigmata and also because of the relativity high concentration of carotenoids in stigmata of latter *Crocus* species, it appeared that *C. haussknechtii* stigmata could be used as a rich source of saffron. Since this crocus is grown in rocky lands, which are often not rich in nutrients, its cultivation in poor soils will yield more quantities of saffron.

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RESEARCH PAPER

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Fault Detecting Intelligent System for Baker's Yeast Fermentation

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Baker's yeast fermentation consists of a number of upstream and downstream processes and is sensitive to contamination, composition of medium, changes in process parameters like pH, temperature and aeration. Any deviation from optimum process condition or any leakage or breakdown in process equipment may lead to huge financial loss. The main problems are maintenance of sterility, avoiding equipment faults and thus keeping maximum production rate. An intelligent diagnostic system for prompt detection of any malfunction of such process will be helpful. Intelligent diagnostic systems have been extensively studied by many research workers. The underlying concept of such a system is to find a collection of disorders (causes) that can be the best explanation for the present set of symptoms (manifestation), which may be considered as a deviation from normal behaviour. The reverse process of reasoning to reach the conclusion without using any deductive rule is known as 'abductive reasoning' A prototype expert system for diagnosis of Baker's yeast fermentation was developed on the expert system shell Knowledge Engineering System.HT (KES.HT) based on abductive reasoning.

Keywords : Fault detection, Intelligent system, Fermentation, Baker's yeast, KES.HT software.

The way of reaching the most plausible set of disorders starting from the available manifestations, without using any heuristic rules is known as 'abductive reasoning' (Charniak et al. 1990). Usually, human diagnostic process is based on this sort of reasoning. An abductive problem is represented by different research workers (Bylander 1991; Peng and Reggia 1990; Josephenson et al. 1987; Reiter 1987; Kakas et al. 1993).

The process starts from collecting all available information (symptoms/manifestations) for a present malfunction (disorder), as soon as a new set of symptoms are available. The problem is to find a collection of disorders, which can be the best explanation covering all symptom set. This approach is much practical and generalized in designing an intelligent problem solver for fault detection and diagnosis.

Baker's yeast fermentation (Reed 1982) consists of five aerobic fermentation stages in a medium containing molasses, urea, phosphoric acid, minerals and vitamins along with downstream process. The fermentation is carried out at a pH between 4 and 5, a temperature between 28-30°C for periods 7-20 h. Different types of faults may occur during both upstream and downstream processes. These faults may lead to huge wastage and poor quality of product that can be restricted with prompt and proper diagnosis by an intelligent expert system (Quantrile and Liu 1991).

The present prototype expert system based on the shell (Quantrile and Liu 1991) KES.HT (Anon 1988; Peng and Reggia 1990) follows abductive reasoning to focus the particular area of fault detection of the Baker's Yeast fermentation processes.

Materials and Methods

Problem definition

Baker's yeast fermentation process (Reed 1982) is a sequence of aerobic fermentations in which 'pitching yeast' are grown in a medium containing molasses, urea, phosphoric acid, minerals and vitamins. The fermentation process is divided into upstream and downstream processes. There are five fermentation stages (D1, D2, D3, D4, D5) in the upstream process. Among downstream processes, there are centrifugal separation of yeast cells, refrigerated storage and filtration of yeast cream, extruding, mixing, packaging and storing. The plant itself consists of jacketted vessels, fermenters, pipelines for fluids, slurry, steam and air valves, compressors, centrifuges, plate and frame filters, pumps etc. Like any other fermentation, the process is sensitive to contamination, change in temperature, acidity (pH), composition of the medium, aeration etc. Any perturbation from optimum conditions, any leakage or breakdown in pipe lines or equipments during the process in progress may lead to huge wastage or poor quality of the product. Any such faults either in upstream or downstream may even destroy the actual purpose of fermentation. In such a process, an intelligent system for prompt detection of the proper fault or group of faults based on the manifestations provided will be helpful in the absence of a human expert.

Solving abductive problem

As presented by Peng and Reggia (1990), a diagnostic problem can be viewed as a mapping from the set of all sub-sets of manifestation of a system to the set of all sub-sets of possible disorders such that each of these sub-sets can be considered as explanation for the corresponding observation set.

The problem environment can be thought as a 4-tuple<D, M, C, M^+>, where, D = $\{d_1, d_2 \ldots\ldots d_n\}$, a finite non-empty set of disorders, where each disorder 'di' has some effects in the set of manifestations (m_j), (Fig. 1a). M = $\{m_1, m_2 \ldots\ldots m_k\}$. A finite non-empty of observations, each manifestation 'm_j' in M can be considered as the effect of some disorders represented by a small sub-set called causes (m_j) in D, (Fig. 1 a). M^+ is the set of present manifestations, represented as the input of the knowledge-based system.

C⊆, the certesian product D X M, represents a set of pairs, (d_i, m_j) for each distinct disorder 'd_i' in D and each distinct manifestation 'm_j' in M.C, is interpreted as the 'causation' i.e., the disorder 'd_i' can cause 'm_j' the manifestation (Fig. 1 b).

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A subset 'E' of D is said to be an explanation of M^+ for a problem P = <D, M, C, M+> (Fig. 1 c), if and only if E covers M^+ and E satisfies the following conditions,

i) The covering requirement i.e., all present manifestations must be covered; ii) the parsimony requirement, which means that the covering must be minimum i.e., the explanation with the smallest cardinality and iii) the explanation must consist of disorders only.

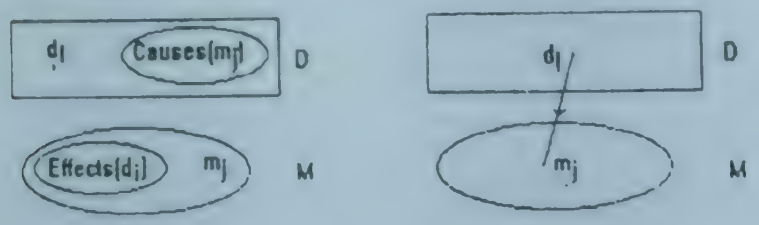

Fig1. (a)

Fig1. (b)

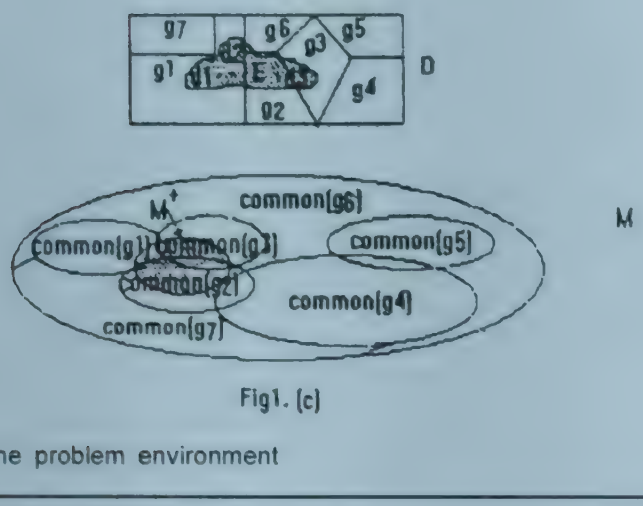

Fig1. (c)

Fig. 1. The problem environment

Among all plausible explanation for a present manifestation set, the authors gave preference to only those explanations having minimum cardinality and non-redundancy. The term "non-redundancy" specifies the criteria of the explanations covering all present manifestations in such a way that by removing any disorder from the explanation set, the covering criteria will not be satisfied.

Based on the above strategy, a domain independent software program, KMS.HT (commercial name KES.HT) was developed by Peng and Reggia (1990) as a tool for solving abductive problem. The term "HT" stands for 'hypotheses and test' circle.

Knowledge in KES.HT follows a frame-based mechanism. There are no direct IF-THEN rules like statements to represent knowledge, instead, a grouping of properties called 'frame' for any given object, situation or process is described. Each description consists of a collection of statements related to the domain (Peng and Reggia 1990). In this situation, a group of manifestations is categorized hierarchically according to a particular fault. The faults are also classified in seven major fault groups, termed 'Generator' (Peng and Reggia 1990) consisting of certain specific faults. Table 1 represents the fault tree of the system in terms of generators (g_1, g_2, g_3, g_4, g_5, g_6, g_7).

In this shell, the user creates a file containing the description for each disorder in terms of manifestation. It causes in a formatted form known as 'assertion' which is represented as <attribute><relation><value[elaborate]>.

TABLE 1. THE FAULT TREE

		Upstream Faults			Downstream faults	
Improper sterilization & sanitation (g_1)	Leakage (g_2)	Improper feeding (g_3)	Breakdown (g_4)	Misc. (g_5)	Mechanical (g_6)	Microbiological (g_7)
Wort (d_1)	Cooling coil (d_{13})	Excess feeding of sugar (d_{20})	Boiler (d_{24})	Jammed cooling jacket/ coil (d_{26})	Separator speed failure (d_{30})	Improperly chlorinated wash water (d_{34})
Cultural vessels/ fermenters (d_2)	Air line (d_{14})	Low feeding of sugar (d_{21})	Blower (d_{25})	Excess steaming of wort (d_{27})	Hydraulic press failure (d_{31})	Infected filter cloth (d_{35})
Airline (d_3)	Steam valve (d_{15})	Low feeding of urea (d_{22})		Faulty medium composition (d_{28})	Disp. pump failure (d_{32})	Improperly cleaned mixing machine (d_{36})
Airfilter (d_4)	Transfer line (d_{16})	Insufficient aeration (d_{23})		Water retention in cooling jacket (d_{29})	Faulty cream collector temp. (d_{33})	Improperly cleaned packing machine (d_{37})
Transfer line (d_5)	Molasses line (d_{17})					Improperly cleaned storage room (d_{38})
Molasses line (d_6)	Culture vessel (d_{18})					
Seed collector (d_7)	Air filter (d_{19})					
Growth in vent pipe (d_8)						
Infiltration of slude into vessel (d_9)						
Infected previous stage (d_{10})						
Improper chlorination of start water (d_{11})						
Infection during change in air line (d_{12})						

KES.HT reads those descriptions and construct a casual network from them. This network is then combined with programs for parsimonious covering and interfacing to users. This has the following steps :

Step 1 : Starting to hypothesize and test process.

When a set of manifestations is given to the system, HT selects one at a time and creates a set of hypotheses, which may be considered as its cause. This initial hypotheses set is called the focus.

Step 2 : Explaining manifestations.

When the next manifestation is incorporated into the system, all the causes of the new manifestation are added into the existing hypotheses set to form new hypotheses capable of explaining all known manifestations including the new one. This time, if any hypothesis from the focus is not capable to explain the chosen manifestation, HT removes it from the focus. If one or more hypotheses remain in the focus, then the manifestation is explained and becomes the current manifestation. This process continues until either all of the pending manifestations are explained or all of the hypotheses are eliminated from the focus.

Step 3 : Question generation and termination process.

Whenever there are no pending manifestations, HT checks for undetermined attributes in a disorder in one of the current hypotheses. To select the next query, the system extracts from the description of the active disorder of the first attribute, whose current value is not known in an assertion. From the candidate attributes, the one that appears in the largest number of description of the active disorder is then selected to form the basis of the next question.

Each time, when a question has been answered by the user, a sequential hypotheses-test cycle begins. This process continues until none of the active disorder's description contains assertion of unknown attributes for reaching a solution.

The HT process stops, when there are not any pending manifestations to explain and not any undetermined attributes mentioned in the description of any disorder in focus.

As the processing terminates, the system organizes the disorder involved in these hypotheses into a collection of several groups of different disorders that implicitly represent a set of hypotheses in the solution.

Results and Discussion

Knowledge structure in KES.HT for the actual diagnostic problem : The actual fermentation is based on the following factors :

Fermentation process : whether upstream or downstream.

Fermentation stages : D1, D2, D3, D4, D5 in the upstream process and centrifugal separation of yeast cells, refrigerated storage and filtration of yeast cream, extruding, mixing, packaging and storing in the downstream process.

Value of pH : whether it is 'above 5', 'below 4' or 'In between 4 and 5'.

Apart from the above factors, smell, foam, infection, gpl, air sterility of the media, presence of foul matters, molasses loss, broth temperature, caramel, steam consumption and pressure, rotameter reading, presence of microorganism, balling, steam, wort chilling time, residual sugar concentration, yeast colour etc. can detect the presence of any anomaly during the fermentation process.

Based on these factors, any deviation from the normally observed symptom can be explained, as it occurs due to certain malfunctions, which can be considered as the direct cause.

After consultation with a group of experts on yeast fermentation, the faults generally occurring are classified basically into two classes, Upstream and Downstream faults. These two classes are again classified into seven major fault groups such as, (1) Improper sterilization and sanitation, (2) leakage, (3) Improper feeding and aeration, (4) Breakdown, (5) Miscellaneous, (6) Mechanical and (7) Microbiological. Each major fault group may contain a set of specific sub-faults as shown in Table 1. As the fault hierarchy is established, all possible manifestations/symptoms may be seen as shown in Table 2. Some of the symptoms also contain sub-symptoms, which are detected in a question-answer session from the user on selection of the particular symptom.

To represent the symptom-disorder set in a frame-based structure (generator), all possible manifestations due to each disorder are collected as 'attribute' set. Table 3 represents such a frame. The system interacts with the user by displaying a set of queries. For each query, the user is presented with a list of possible answers that may be selected by using appropriate function keys provided by the shell (Table 4).

In the first stage, it collects all the necessary information regarding the stage of the fermentation process. Next from a

TABLE 2. THE SYMPTOM TREE

- pH not between 4 & 5 (m_1)
- Other than typical sweet smell (m_2)
- Persistent foam (m_3)
- Infection microscopically detected (m_4)
  - Lactobacillus (m_{41})
  - Putrefying bacteria (m_{42})
  - Coliform bacteria (m_{43})
  - Airborne bacteria (m_{44})
  - Coccus (m_{45})
  - Fungus (m_{46})
  - Wild yeast (m_{47})
  - Algae (m_{48})
  - Waterborne bacteria (m_{49})
- Unusual gpl (m_5)
  - Falling (m_{51})
  - Increasing (m_{52})
- Increased broth temperature (m_6)
- Unusually high refrigerant requirements (m_7)
- Falling steam pressure (m_8)
- Falling rotameter reading (m_9)
- High residual sugar concentration (m_{10})
- Steam condensate temperature from culture vessel or fermenter is < 95°C (m_{11})
- Air sterility check medium indicator colour is changed (m_{12})
- Fuel water inside tapped process molasses (m_{13})
- Previous stage microbiological report (m_{14})
- Seed smell other than sweet (m_{15})
- Steam condensate temperature from seed collector is <95°C (m_{16})
- High steam consumption during fermentation (m_{17})
- High balling (m_{18})
- Reddish yeast (m_{19})
- Alcohol present (m_{20})

TABLE 3. SYMPTOM-DISORDER FRAME

Present symptoms	Major group of disorders	Possible disorder
m_1, m_2, m_3, m_5, m_{41}, m_{42}	g_1	d_1, d_2
m_1, m_2, m_3, m_5, m_{11}, m_{43}	g_1	d_3
m_1, m_2, m_5, m_{12}, m_{43}, m_{49}	g_1	d_4
m_1, m_2, m_5, m_{12}, m_{43}, m_{45}, m_{46}, m_4	g_1	d_5, d_6
m_1, m_2, m_4, m_5, m_{13}	g_1	d_7
m_1, m_2, m_4, m_5, m_{47}	g_1	d_8
m_1, m_2, m_4, m_5, m_{14}, m_{15}, m_{16}	g_1	d_9
m_1, m_2, m_4, m_5, m_{15}, m_{16}	g_1	d_{10}
m_5, m_6, m_{17}	g_1	d_{11}
m_1, m_2, m_5, m_{43}, m_{48}	g_1	d_{12}
m_1, m_2, m_5, m_{12}, m_{44}, m_{45}, m_{46}	g_2	d_{13}

TABLE 4. A SCREEN DISPLAY

WHAT IS THE STAGE IN PROGRESS ?

1. D_1
2. D_2
3. D_3
4. D_4
5. D_5
6. Separation
7. Collection
8. Filtration
9. Mixing
10. Packing
11. Storing

= ?

SELECT OPTION NO. (1..10) AND PRESS RETURN

given symptom set, the user is asked to select the unusual indication, which may be considered as a deviation from the normally observed one.

Based on the user's answer, the system starts the hypothesize-and-test process to find the possible group of disorder, which can be a possible set of explanation. As a new manifestation is incorporated into the system, all the causes of the new manifestation are added into the existing explanation set to form new hypotheses capable of explaining all known manifestations including the new one. This time, if any hypothesis from the focus is not capable to explain the chosen manifestation HT removes it from the focus. This process continues, until all of the symptoms presented by the user are explained.

Implementation : The abductive expert system shell KES.HT, loaded under the work station 'APOLLO' has an in-built inference mechanism (Quarentile and Liu 1991) with a frame-based architecture.

Knowledge base developed by KES.HT consists of four sections in the following order :

Text : This contains extra information required for clarification.

Attribute : This is the hierarchical representation of the attributes of the objects, situation or process. There are two types of attributes, input and inferred. Input attributes are those whose values can be determined directly by asking the end users asserted in the actions section, read from the communication files or come from the embedded interface Inferred attributes are attributes whose values are usually determined by knowledge sources such as description, default and calculated clauses and external program or by the sources for input attributes.

Example :

Input Attributes

Menu : sgl

(D1, D2, D3, D4, D5, separation, collection, filtration, mixing, packaging, storing)

question : "WHAT IS THE STAGE IN PROGRESS" ?

From this declaration, the system will accept only one menu option as input and store as the value of the stage of the process.

Inferred Attributes

Cause : mlt

Excess feeding [description : sym = increased_broth_temperature, high_residual_sugar_concentration, sys3 = high_balling, alcohol_present, reddish_yeast;] %

Demons : These are event-based actions, which are performed on satisfying a set of conditions.

Example :

Demons :

Micro demon :

when

Sym = infected_colony_under_microscope

then

ask for micro

end when.

%

Action : This section contains the instructions to control the system and to provide user interface.

There are commands for displaying prompt and for setting goal attributes for the inference engine.

Example :

Actions :

display attach purpose of Kb.

message " ", "Type 'C' to begin"

break.

ask for menu.

obtain cause.

message.

" "

"the following are the possible location of faults".

display value of cause.

message " ", "type 'n' for another case or 's' to stop".

%

To validate the performance of the abductive frame-based

TABLE 5. A REAL RUN TIME SESSION

Input		Output	
Attribute	Value	Frame-based	Rule-based
Process stage	D4	1) Excess feeding 2) Insufficient aeration	1) Excess feeding
Symptoms	_other than typical sweet smell		
	_unusual gpl		
	_Increased broth temperature		
	_High residual sugar concentration		
	_Seed smell usual sweet		
	_previous stage microbial report not known		
	_High balling		
	_Low gpl		
	_Alcohol present		

expert system, a rule-based prototype in the shell Level 5 (Anon 1986) has also been developed. Both the prototypes diagnose with the help of symptoms, which were the deviations from normally observed values generally monitored in a commercial Baker's yeast production process. From several case studies, it became evident that the diagnosis with the help of a frame-based abductive intelligent system was more appropriate to practical cases. Using abductive reasoning, the present prototype took care of multiple disorders with complementary alternative combination, which was not available in case of deductive rule-based prototype expert system. This is clear by a real run time session presented in Table 5.

Moreover, knowledge representation in KES.HT followed exactly the same way of human expert's narration i.e., from cause to effect. 'Hypothesize and test' cycle continues, until plausibility of one or more hypotheses passed some given criteria and they were accepted as reasonable explanations for the given findings.

Any parameter other than usually monitored ones had not been included in the symptom list of the knowledge base of the present prototypes. As microbial identification was time consuming, there was not very much dependence on the symptoms like type of microorganism. There were other symptoms by which the fault could be diagnosed. Microbial identification may confirm the fault later on. Actually, current approach would combine on-line data with off-line sample analysis and microscopy.

Conclusions

Still there remains an immense scope for modification and further enrichment of knowledge base by introducing more facts about the process.

Probability of symptoms against a fault as also of occurrence of faults are still to be included in the knowledge base. Inclusion of immediate remedial measure for some typical malfunction will be a more practical approach for this type of system. Scope of augmenting the knowledge base for fault diagnosis in active dry yeast production is also felt.

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RESEARCH PAPER

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Glucoamylase Production by *Aspergillus niger* in Solid State Fermentation with Paddy Husk as Support

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Aspergillus niger CFTRI 1105 produced the highest amount of glucoamylase 875.5 Ug^{-1} DS (3rd day) in a medium containing soy meal powder (200.0 g), paddy husk (460.0 g), mineral solution (20.0 ml) and tap water (180.0 ml). Glucoamylase activity obtained in media containing beef and soy meal powder having same elemental nitrogen level and starch concentration were closer to each other. Optimization of moisture content and spore inoculum size improved glucoamylase production with reduction in production time. Optimized amount of paddy husk to soy meal powder ratio was 7.0:3.0. By optimizing the conditions, production of glucoamylase increased to 1853.7 Ug^{-1}DS on the 2nd day.

Keywords : Solid state fermentation, Paddy husk, Soy meal powder, Glucoamylase, Dry substrate.

Glucoamylase is produced by submerged (Jansz et al. 1977; Ramadas et al. 1996; Kolhekar et al. 1985) and solid state (Oriol et al. 1988; Ramadas et al. 1996; Ghildyal et al. 1985) fermentation (SSF) processes. Solid state fermentation technique is the traditional fermentation method of South-East Asia (Aidoo et al. 1982; Cannel and Moo-Young 1980 a, b; Hasseltine 1977 a,b and is still in use (Ghildyal et al. 1992; Gowthaman et al. 1993) for the production of α-amylase (Ramesh et al. 1993), glucoamylase (Ghildyal et al. 1985), protease (Battaglino et al. 1991: Shanmuganathan et al. 1993) gibberellic acid (Kumar and Lonsane 1989), fibrinolytic enzyme (Tao et al. 1997) and pectinase Ghildyal et al. 1981). Previous reports suggested that wheat bran (Ghildyal et al. 1985; Ramakrishna et al. 1982; Ramadas, et al. 1996) with additional nutrients such as corn meal and minerals was useful for the production of glucoamylase and other enzymes by SSF processes. Wheat bran is not available in Sri Lanka and hence in the present study, rice bran and paddy husk as alternative substrates were used (Pandey et al. 1994 a;b) and / or support (Arasaratnam et al. 1997) for glucoamylase production. Since the use of paddy husk was attractive as a support, it was decided to optimize the culture conditions and formulate a medium for *Aspergillus niger* CFTRI 1105.

Materials and Methods

Materials : Soy and corn flours were prepared by milling soy seeds and corn grains (*Zea mays*. L) purchased from local market. Paddy husk available in the local grinding mill was used. Waxy maize starch was from Stardex, Sweden. Soy meal (Delmage, Colombo) was ground to powder in a domestic grinder. Beef and ox intestines were purchased from a local slaughter house and minced in a domestic mincer.

Microorganism : Aspergillus niger CFTRI 1105 was from CFTRI, Mysore, India. The organism was sub-cultured once in every two weeks on Potato Dextrose Agar (PAD) slants and stored at 4°C (Navaratnam et al. 1996). Whenever the spores were required, they were suspended in 1.0% (v/v) Tween -80 and the number of spores were counted using a haemocytometer.

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Glucoamylase activity : Enzyme activity was determined as described previously (Arasaratnam, et al. 1997) and the activity of glucoamylase is presented as U g^{-1} DS (dry substrate).

Comparison of corn flour, soy flour and soy meal powder: Paddy husk (460.0 g) was mixed with corn flour (medium I) or soy flour (medium II) or soy meal powder (medium III), mineral solution (containing (g^{-1}) $CuSO_4$. $5H_2O$, 0.7 g; $FeSO_4$. $7H_2O$, 0.7 g and $ZnSO_4$. $7H_2O$ in 2N HCl) and tap water (Table 1) in 5 litre conical flasks and sterilized. The pH of the medium was 4.0. The spore suspension (4.5 X 10^7 ml^{-1}, 20.0 ml, in 1.0%, v/v Tween-80) inoculum was added and incubated at 30°C up to 4 days. Enzyme activity and moisture content (Pearson 1976) of the media were measured.

Effect of soy meal powder concentration : Composition of the media prepared with different concentrations of soy

TABLE 1. COMPOSITIONS OF THE MEDIA TO STUDY THE EFFECTS OF CORN FLOUR, SOY FLOUR AND SOY MEAL POWDER

	Medium		
Constituents	I	II	III
Corn flour, g	200.0	-	-
Soy flour, g	-	200.0	-
Soy meal powder, g	-	-	200.0
Paddy husk, *g	460.0	460.0	460.0
Mineral solution **ml	20.0	20.0	20.0
Tap water, ml	180.0	180.0	180.0
Wet weight of substrate, g	200.0	200.0	200.0
Dry weight of substrate, g	176.0	180.0	180.0
Total activity, U	57200.00	122958.0	157590.0
Activity, U g^{-1} substrate	325.0	683.1	875.5
N/C ratio	0.12	0.58	1.57

The media were taken in 5 litre conical flasks

*Paddy husk (460 g) was soaked in water and suction-dried before mixing

**Mineral solution contained (g^{-1}) $CuSO_4$. $5H_2O$, 0.7: $FeSO_4$. $7H_2O$, 0.7 and $ZnSO_4$. $7H_2O$, 0.7 dissolved in 2M HCL

The ratio between dry paddy husk and wet substrate was 7.0:3.0

TABLD 2. MEDIA CONTAINING DIFFERENT CONCENTRATIONS OF SOY MEAL POWDER

Constituents	Medium III	IV	V	VI	VII	VIII
Soy meal powder, g	200.0	-	50.0	100.0	150.0	250.0
WMS[a]	-	68.7	51.6	34.4	17.2	-
Paddy husk, g	460.0	160.0	237.1	313.6	390.1	583.3
Mineral solution, **ml	20.0	6.9	10.2	13.4	16.7	25.0
Tap water, ml	180.0	62.1	91.8	120.6	150.3	225.0
Wet weight of substrate, g	200.0	68.7	101.6	134.4	167.2	250.0
Dry weight of substrate, g	180.0	59.1	89.4	119.2	149.8	225.0
Total activity, U	157590.0	1436.1	9306.5	34389.2	63005.9	44945.0
Activity, U g^{-1} substrate	875.5	24.3	104.1	288.5	420.6	644.2
N/C ratio	1.57	-	0.40	0.79	1.18	1.57

*The media were taken in 5 litre conical flasks
**Composition of mineral solution was as given in Table 1
[a]Starch contents of soy meal powder and WMS were 30.0 and 86.3 %, respectively
Substrate to mineral solution ratio was 10.0:1.0 and the mineral solution to tap water ratio was 1.0:9.0

TABLE 3. DIFFERENT NITROGEN SOURCES CONTAINING MEDIA

Constituents	Medium III	IX	X
Soy meal powder, g	200.0	-	-
Beef, g	-	449.5	-
Intestine, g	-	-	726.5
Waxy maize starch, g	-	68.7	68.7
Paddy husk, *g	460.0	1209.1	1855.5
Mineral solution **ml	20.0	51.8	79.5
Tap Water, ml	180.0	-	-
Wet weight of substrate, g	200.0	518.2	795.2
Dry weight of substrate, g	180.0	171.5	240.5
Total activity, U	157590.0	147592.9	136813.9
Activity, U g^{-1} substrate	325.0	683.1	875.5
N/C ratio	875.5	860.0	568.4

The media were taken in 5 litre conical flasks
**Composition of the mineral solution was as given in Table 1
Protein contents of soy meal powder, beef and intestine were 47.2, 21.0 and 13.0 %, respectively.
Water content of beef and intestine was considered as 75 % and tap water was not added.
The ratio between dry paddy husk and wet substrate was 7.0:3.0

meal powder and paddy husk by equalizing the starch content with waxy maize starch are given in Table 2. The ratio between the substrate and paddy husk was 3.0:7.0.

Effect of different nitrogen sources : Media containing soy meal powder (Control medium, medium III), beef or intestine (having same elemental nitrogen level) were prepared (Table 3). Starch contents of media containing beef and intestine were equalized as above.

Effect of initial moisture content of the medium : Moisture content of medium III was varied form 35.0 to 78.0% by varying the amount of tap water, while taking the same amount of mineral solution (20.0 ml).

Effect of spore inoculum size : To medium III with optimized amount of moisture content, spore suspension inoculum from 4.5×10^6 to 4.5×10^9 ml^{-1} (20.0ml, in 1.0%, v/v Tween-80) was added and incubated at 30°C up to four days.

Effect of paddy husk to soy meal powder ratio : Paddy husk to soy meal ratio was varied from 0.0:10.0 to 10.0:0.0 (Table 4). The media were inoculated with optimized amount of spore inoculum.

Results and Discussion

Comparison of corn flour, soy flour and soy meal powder: Glucoamylase produced in the medium containing soy meal powder (medium III) was the highest, followed by soy flour and corn flour (Table 1). In all three media, proteins, carbohydrate and nitrogen contents were not maintained and maximum activity was obtained on the 3rd day. Protein contents of soy meal powder, soy flour and corn flour were 47.2, 31.2 and 9.0 %, respectively. Soy meal was a by-product obtained, while soy milk was prepared and it was richer in proteins than soy flour. Thus, the increased glucoamylase production in these media must be due to the high content of proteins of soy meal powder than soy and corn flours. However, if the proportion between N/C ratio of media I and II were compared, they were not related to the proportion between the glucoamylase activities obtained in the two media (Table 1). Corn steep liquor is reported to be better than peptone in glucoamylase production (Pandey et al. 1994; Moo-Yong et al. 1983). Thus, it was concluded that the enzyme production not only depended on N/C ratio but also on the organisms, product to be formed and culture conditions (Wang et al. 1974; Navaratnam et al. 1995).

Effect of soy meal powder concentration : To media containing different concentrations of soy meal powder, waxy maize starch (a purified insoluble starch containing 86.3 %; w/w starch) was added to equalize the starch content to that in medium III, except in medium VIII (Table 2). Protein and nitrogen contents of the media were not maintained. In all media, maximum glucoamylase was produced on the 3rd day. Glucoamylase production was negligible in medium that contained no soy meal powder (medium IV, Table 2). Glucoamylase produced in medium V was 4.3 times more

TABLD 4 COMPOSITION OF THE MEDIA WITH DIFFERENT RATIOS OF PADDY HUSK TO SOY MEAL POWDER

Constituents	Medium (Paddy husk: Soy meal powder)						
	XI (0.0:10.0)	XII (3.0:7.0)	XIII (5.0:5.0)	XIV (6.0:4.0)	III (7.0:3.0)	XV (8.0:2.0)	XVI (10.0:0.0)
Soy meal powder, g	200.0	200.0	200.0	200.0	200.0	200.0	-
Paddy husk, **g	-	85.7	200.0	300.0	460.0	800.0	500.0
Mineral solution, *ml	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Tap water, ml	180.0	180.0	180.0	180.0	180.0	180.0	180.0
Wet weight of substrate g	200.0	200.0	200.0	200.0	200.0	200.0	-
Dry weight of substrate g	180.0	180.0	180.0	180.0	180.0	180.0	460.0
Total activity, U	100170.0	150210.0	194458.0	252108.0	333666.0	319500.0	3128.0
Activity, U g^{-1} substrate	5565.0	835.5	1081.1	1400.6	1853.7	1775.0	6.8

Media XI and XII were taken in 2 litre and III, XIII, XIV, XV and XVI in 5 litre conical flasks

*Composition of mineral solution was as given in Table 1

**Paddy husk was soaked in water and suction-dried before mixing.

than that in medium IV (Table 2). Increase in added soy meal powder from 0.0-200.0 g (in media IV, V, VII and III, respectively) increased glucoamylase production and was highest in medium III. The results showed that the total glucoamylase activity obtained in medium III was 12645.0 U more than that obtained in medium VIII. It is interesting to note that additional 50.0 g of soymeat powder was used in medium VIII. Wheat bran is the commonly used substrate for enzyme production in SSF (Ghildyal et al. 1985; Ramakrishna et al. 1982). Rice bran has also been reported to be an alternative for glucoamylase production (Pandey et al. 1994 a; Arasaratnam et al. 1997). However, inclusion of paddy husk helped to yield more glucoamylase production than rice bran, by efficiently utilizing the substrate (Arasaratnam et al. 1997). Thus, paddy husk was used as support with soy meal powder. Reduced glucoamylase production in medium IV, when compared to all other media (Table 2) indicated the importance of nitrogen source. It may be noted that waxy maize starch does not have proteins, (Arasaratnam 1989). Glucoamylase produced (g^{-1} DS) in medium III was 1.4 times more than that in medium VIII, where N/C ratio of the two media were same (1.57) (Table 2). These results indicated that the enzyme production not solely depended on N/C ratio (Pandey et al. 1994 b) but also on the total amount of nitrogen source used.

Effect of different nitrogen sources : Glucoamylase produced in media containing soy meal powder and beef (media III and IX, Table 3) were very close to each other, while that produced in medium X was 64.9 % of that in medium III. In all three media, maximum glucoamylase was produced on the 3rd day. Soy is generally mentioned as "poor man's beef" (Wickramanayake 1987). Here too, the quality of soy protein is comparable to beef on enzyme production. Decrease in activity in medium X could be due to the high content of fibrous proteins in intestine and might not be the substrates for proteases produced by *A.niger*. Beef being expensive, it was practically not feasible to use beef in enzyme production. However, intestine could be used after optimizing the amount.

The amount of mineral solution used was 10.0 % of the total wet weight of all the substrates and the mineral solution to tap water ratio was 1.0:9.0. Required amount of paddy husk was soaked in tap water and suction, dried before mixing to the substrate and then autoclaved. Soy meal powder contained 30.0% (w/w) starch and 47.2 % (w/w) total proteins.

Effect of moisture content of the media : Maximum glucoamylase activity was obtained on the 2nd day in media containing moisture levels between 35.0 and 60.0 %, while on the 3rd day, the activity was maximum in the media having moisture contents above 60.0 % (Fig. 1). Increase in moisture level from 50.0 to 55.0 % has resulted in 1.64-fold increase in glucoamylase production (Fig. 1). Even in solid state fermentation, water must be available for microbial activities (Huang et al. 1985). However, moisture requirement is governed by nature of the substrate type of end product and the microorganism (Lonsane et al. 1985). Moisture level in medium also determines the vegetative growth and sporulation of an organism (Wang et al. 1975). Therefore, the initial moisture content required for *A. niger* CFTRI 1105 in the formulated medium was studied. Increase in moisture content above 65.0 % delayed the maximum enzyme production time, which was similar to that generally observed in the submerged fermentation with *Aspergillus niger* CISIR N4 (Ramadas, et al; Jansz et al. 1977). When the moisture level was increased, the settling of the solid materials from the liquid medium was observed and this would have led to decreased porosity, loss of particle structure, reduction of gas volume, decreased gas exchange, lowered oxygen transfer due to decreased diffusion (Lonsane et al. 1985 ; Nishio et al. 1979 ; Silman et al. 1979 ; Feniksova et al. 1960). Increased moisture level may be aiding the proteolytic activity of acid protease produced by this organism (Results are not reported). Similarly, low moisture levels also may lead to sub-optimal growth, lower degree of substrate swelling, reduced solubility of the nutrients present in the solid substrate and high water tension (Wang et al. 1974 ; Lindenfeleser and Ciegler 1975; Feniksova et al. 1960 ; Zadrazil and Brunnert 1981). Optimum moisture level for SSF of different organisms around 55.0 % has been reported (Ramesh and Lonsane 1990; Huang and Woodams 1987; Tao et al. 1997; Battiaglino et al. 1991). Therefore, it is essential to maintain the moisture level at a narrow range between 50.0-60.0 % to accelerate and increase the glucoamylase production by *A. niger* CFTRI 1105.

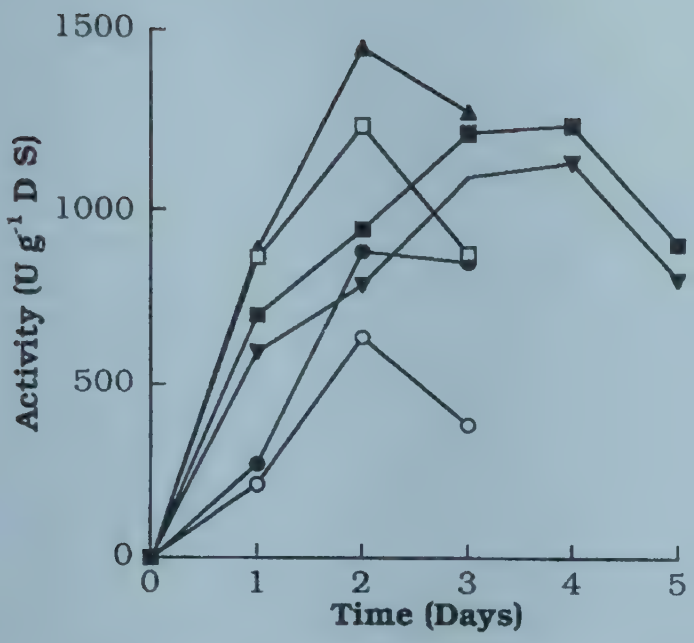

Fig. 1. Effect of initial moisture level on glucoamylase production from *Aspergillus niger* CFTRI 1105 by solid state fermentation at pH 4.0 and 30°C. (○) 35.0, (●) 50.0, (▲) 55.0, (❑) 60.0, (■) 65.0 and (▼) 78.0%

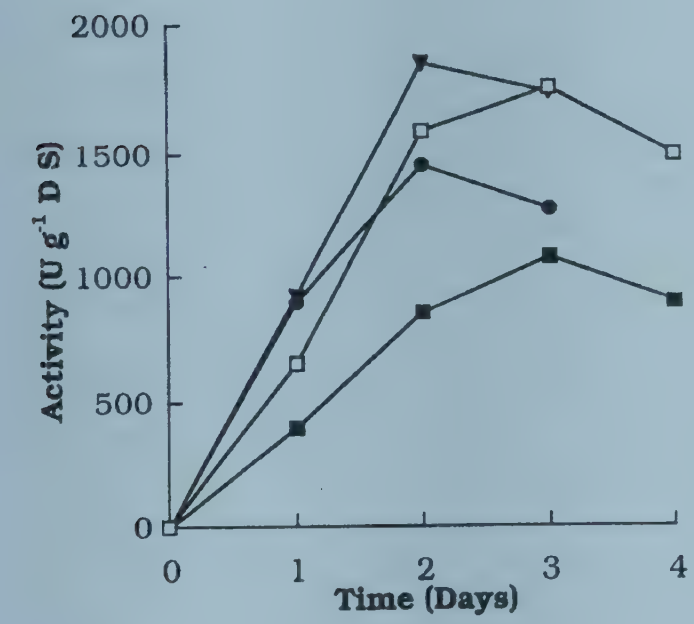

Fig. 2. Effect of spore number in inoculum on glucoamylase production from *Aspergillus niger* CFTRI 1105 by solid state fermentation at pH 4.0 and 30°C. (■) 4.5 x 10^6, (●) 4.5 x 10^7, (▼) 4.5 x 10^9 spore ml^{-1}

Effect of spore inoculum size : Optimization of the number of spores in the inoculum is essential. Linear relationship between the spore number in the inoculum and the cultivation time has been reported (Wang et al. 1974; Oriol et al. 1988 ; Huang et al. 1985 ; Gervais and Sarrette 1990 ; Shanmuganathan et al. 1994; Tao et al. 1997). When the spore number was increased from 4.5 x 10^7 to 4.5 x 10^8 ml^{-1}, maximum enzyme production was obtained on the 2nd day, while with 4.5 x 10^5 and 4.5 x 10^9 spores ml^{-1}, maximum activity was obtained on the 3rd day (Fig. 2). These results indicate that the number of spores inoculated should be optimized and having higher or lower levels of spores may affect and delay the enzyme production. Spore number of 4.5 x 10^8 ml^{-1} was selected for the following experiments.

Effect of paddy husk to soy meal powder ratio : In all media, maximum glucoamylase activity was obtained on the 2nd day. Medium XVI, contained husk alone gave 6.0 Ug^{-1} dry husk (Table 4). In contrast, in medium XI with soy meal powder alone, maximum activity obtained was 556.6 U g^{-1} DS. With a change in paddy husk to soy meal powder concentration ratio form 0.0:10.0 to 7.0:3.0, glucoamylase production increased. Highest glucoamylase activity was obtained in medium III, followed by medium XV (Table 4). The activity obtained in medium III was 2.22 times more than that in medium XIII. Similarly, the activity obtained in medium III was 3.33-fold higher than that in medium XI. In the presence of paddy husk, nutrients in the substrate are beter utilized (Arasaratnam et al. 1997). This could be due to the efficient air circulation and nutrient diffusion caused by the intetial space created by paddy husk. The husk also must be increasing the surface area for the spores to germinate and mycelia to grow, making them to get access to the added nutrients easily. This is supported by the observation that the mycelial growth was significantly seen over the husk, when observed under microscope. Since the glucoamylase activity obtained in the medium (medium XVI) having paddy husk alone was negligible, it was concluded that the husk was not contributing essential nutrients for the fungus and it was acting as an efficient support. Free movement of air and hence, better oxygen transfer can be achieved by using porous, coarsely granulated or fibrous substrate (Huang et al. 1985; Battaglino et al. 1991), a lower thickness of substrate layer, high level of air space in the substrate and use of perforated trays, a fermentor vessel with a wore mesh cloth surface, shaking or agitating the substrate or rotating the fermentor (Lonsane et al. 1985; Ramesh and Lonsane 1990; Linderfelser and Ciegler 1975). In present studies, the paddy husk as a support was selected (Arasaratnam et a. 1997) instead of a porous, coarsely granulated or fibrous substrate. Battaglino et al (1991) have used rice hull and rice bran (7.0:3.0) and obtained highest protease activity. The present results have shown that paddy husk to soy meal powder in the ratio of 7.0:3.0 is the best to get a yield of glucoamylase per gram of dry substrate.

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RESEARCH PAPER

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Analysis of Menthol Content in *Pan Masala* Samples Collected from the State of Uttar Pradesh

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According to Prevention of Food Adulteration Act of India, 0.1% of menthol in *Pan Masala (PM)* samples is permitted. *Pan Masala* is a mixture of arecanut, catechu, lime, cardamom and unspecified flavouring agents with or without tobacco. It is being consumed as a substitute for betel quid. Among 622 samples collected from the State of Uttar Pradesh, India, only 9.32 % samples showed the level of menthol below 0.1 %. Almost 89 % of *sada* and 94 % of *zarda PM* samples exceeded the permissible limits of menthol. In village and city markets, almost 89 and 93 % *PM* samples showed higher levels of menthol than the PFA limits, respectively. Only 3.3 and 15.4 % of packed and non-packed *PM* samples were within the permissible limits, respectively. Samples collected from one district of the State showed menthol within the permissible limit, while those collected from remaining 62 districts showed the average values above 0.1 %. The study showed that 6 brands of *PM* contained menthol within the permissible limit, while the remaining 44 brands exceeded the prescribed limits of menthol. It was suggested that Goods Manufacturing Practices should be adopted by those manufacturers whose products had the menthol concentration, exceeding the permissible limit. Menthol content was found to be more than 0.1 % in samples drawn from 5 zones of the State.

Keywords : Menthol, Pan Masala, Packed, Non-packed, *Sada* (without tobacco), *Zarda* (with tobacco), Village and city markets Samples, Districts, Zones.

Menthol is a terpenoid compound extracted from flowering parts of Mentha sps. of Labiate family. Menthol causes chronic conjugated hyperbilirubinimea (Arias 1962) and hypersenitivity with urticaria, flushing and headache (Papa and Shelly 1964). Menthol has been shown to produce psycholeptic effect in mouse. However, its psychrotropic activity is very weak, when administered orally (Murthy and Prema 1979). Oral administration of menthol (200-800 mg/kg) to rats showed significant increases in absolute and relative liver weight with vascularization of hepatocytes, (Thorup et al. 1983). It causes colonic spasm due to muscle relaxant activity and acts as a calcium antagonist (WHO 1976 ; Leicester and Hunt 1982) and non-thrombocytopenic purpura (Highstein and Zeligman 1951). Excessive consumption of menthol also causes bradycardia, ataxia, mental instability, thickened tone and instant death of children (Luke 1962; Martindale Extra Pharmacopoeia 1972, 1989).

In India, it is widely used in the preparation of *Pan Masala (PM)*, which is consumed by large population (Rao et al. 1983; Bhatia et al. 1991; Murthy et al. 1991). *Pan Masala* is a dry mixture of areca nut, catechu, lime, cardamom and unspecified flavouring agents with or without tobacco and is commonly marketed under different names (Adhvaryu et al. 1991). The one without tobacco refers to as *sada PM,* while the other with tobacco is known as *zarda PM.* It has all the ingredients of betel quid minus betel leaf with some additional additives. Since Prevention of Food Adulteration Act of India 1954 (1997) has prescribed a level of 0.1 % menthol addition in *PM,* the scenario of usage of menthol in *PM* samples collected from the State of Uttar Pradesh, India was investigated.

Materials and Methods

Chemicals and reagents : *p*-dimethyl amino benzaldehyde (Rearal, Budapest, Hungary), sulphuric acid (Qualigen, Mumbai, Excellar; 98.08 % purity), menthol (Central Drug House, New Delhi, grade with 99 % purity) and absolute alcohol (99.5 % Bengal Chemicals, Kolkata) were purchased and used in this study.

Collection of samples : Five samples of *PM* were procured from one rural market and one city market of each 62 districts of the State of Uttar Pradesh through the State Planning Institute (Economics and Statistical Division, Government of Uttar Pradesh, Lucknow). Thus, 10 *PM* samples per district were collected from a total of 62 districts of the State except in one district, where 12 samples were collected, making a total of 622 samples. From each market, out of 5 samples, 3 were *sada PM* and 2 were *zarda PM* (tobacco-mixed) samples.

Estimation of menthol : The method of menthol estimation in *PM* samples is essentially the same as described by Kannan et al (1997). The steam is generated and passed over the sample of *PM*. Since menthol is a volatile compound, steam was expected to evaporate it. The vapours of menthol were condensed and collected in the receiving flask containing a known volume of ethanol. A 0.5 ml of distillate was added with 0.5 % of *p*-dimethylamino-benzaldehyde in concentrated sulphuric acid: water mixture (1.6:1, v/v) and shaken carefully. The mixture was kept for 2 min on boiling water bath and cooled. The red colour formed was read at 550 nm.

Statistical analysis' : The significant differences in the levels of menthol in *zarda* and *sada PM* in five different zones of the State were carried out by two way analysis of variance (Snedecor and Cochran 1967), considering menthol concentration as dependent variable and type of preparations and different zones of the State an independent variable. The inter group comparisons were done by calculating least significant differences in mean values. Similar analysis was carried out in packed vs. non-packed samples, villages vscity market samples in different regions of the State.

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Results and Discussion

The data on menthol contents of *PM* samples are shown in Table 1. Out of 622 samples, 58 samples (9.32 %) had menthol in the range of 0.0-01.0 mg/g, while a maximum of 386 samples (62 %) showed a range of 2.1-6.0 mg /g. Further, only 3 samples (0.5%) fell within the highest range of 10.1-20.0 mg/g (Table 1). These results indicated that only 9.32 % samples had the permissible limit of menthol added to *PM* (0.1 %) as described under the Prevention of Food Adulteration Act of India 1954 (1997).

The variations in menthol contents in *sada* and *zarda PM* samples are depicted in Table 1. It is interesting to note that 11.26 % of *sada PM* samples were within the prescribed limits of menthol addition as compared to 6.20 % of *zarda*-mixed samples. Though, 48.6 % *sada PM* samples showed the presence of menthol in the range of 1.1-4.0 mg/g with respect to 34.5 % in *zarda PM* samples, 59.3 % of *zarda PM* samples showed the menthol content in the range of 4.1-20 mg/g as compared to 40.1 % of *sada PM* samples (Table 1). Thus, it is quite likely that the habitual consumers of *zarda PM* samples may be at risk due to excessive menthol intake when compared to *sada PM* consumers.

The menthol contents in *PM* samples collected from village and city markets are shown in Table 1. In village market, 11.4 % samples of *PM* had menthol contents below the prescribed limit, while 7.3 % of city market samples were in the same range. The trend due to addition of menthol in *PM* samples in city versus rural markets was quite similar. The similarity in the trend due to addition of menthol in *PM* samples in city and village markets suggested that majority of the manufacturing units were the usual suppliers of pouches of *PM* to the city and rural markets.

Table 1 also shows the contents of menthol in packed and non-packed samples of *PM*. Only 3.3 % packed samples were within the prescribed limit, while 15.4 % of non-packed samples fell in this range. On the contrary, 40.6 % packed *PM* samples showed a range of 4.1-6.0 mg menthol/g as compared to only 22.3 % non-packed samples in the same range.

The menthol contents in *PM* samples in different districts of Uttar Pradesh are shown in Fig. 1. Only one district of Uttar Pradesh showed that the menthol content of *PM* samples was

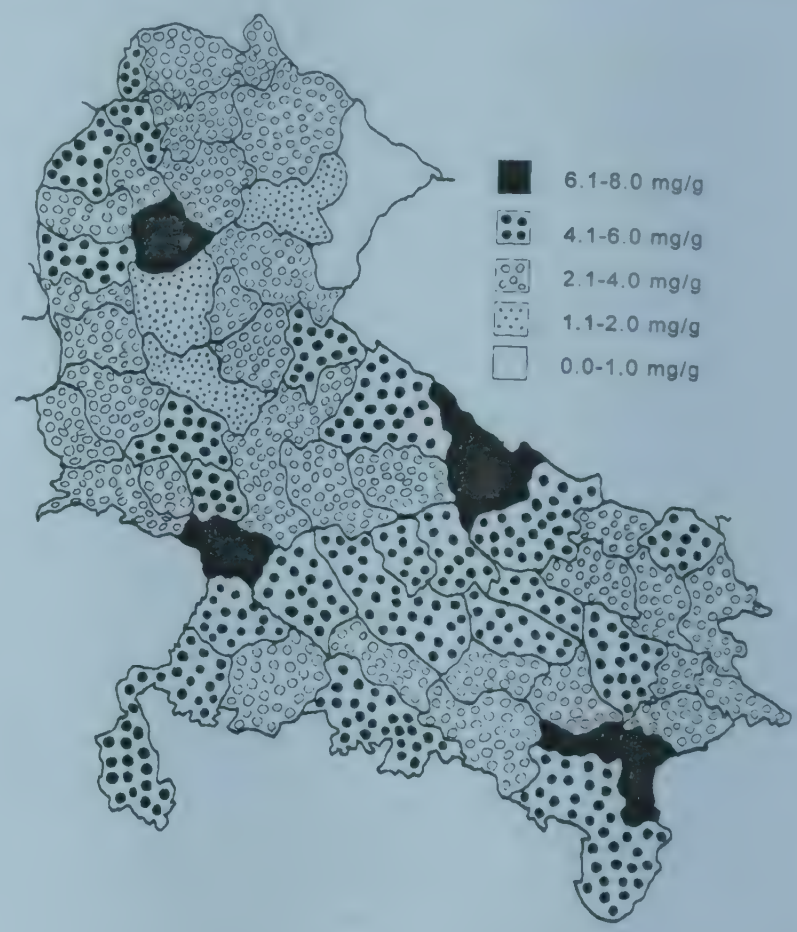

Fig. 1. Menthol content in *Pan Masala* samples in different districts of Uttar Pradesh

TABLE 1. GROUPWISE BREAKUP OF SURVEY DATA ON MENTHOL CONTENT OF *PAN MASALA*

Group	Number, %	Range of menthol mg/g, *pan masala*						
		0.0-1.0	1.1-2.0	2.1-4.0	4.1-6.0	6.1-8.0	8.1-10.0	10.1-20.0
Total	Number	58.0	71.0	193.0	193.0	85.0	19.0	3.0
n = 622	(%)	9.3	11.4	31.0	31.0	13.7	3.1	0.5
Type								
Sada	Number	41.0	48.0	129.0	100.0	37.0	7.0	2.0
n = 364	(%)	11.3	13.2	35.4	27.5	10.2	1.9	0.6
Zarda	Number	16.0	25.0	64.0	95.0	45.0	12.0	1.0
n = 258	(%)	6.2	9.7	24.8	6.8	17.4	4.7	0.4
Location								
Village	Number	35.0	33.0	99.0	90.0	38.0	9.0	3.0
n = 307	(%)	11.4	10.8	32.3	29.3	12.4	2.9	1.0
City	Number	23.0	38.0	94.0	105.0	46.0	9.0	-
n = 315	(%)	7.3	12.1	29.8	33.3	14.6	2.9	-
Marketed as								
Packed	Number	10.0	16.0	81.0	123.0	62.0	11.0	
n = 303	(%)	3.3	5.3	26.7	40.6	20.5	3.6	-
Non-packed	Number	49.0	55.0	112.0	71.0	22.0	7.0	3.0
n = 319	(%)	15.4	17.2	35.1	22.3	6.9	2.2	0.9

within the permissible limits, while the remaining 62 districts exceeded the normal limits of menthol addition. As many as 53 districts showed a 2.1-6.0 mg/g range of menthol in *PM* samples.

The mean value ranges of menthol conten in *sada* vs *zarda* samples and packed vs non-packed samples in 5 different zones of Uttar Pradesh are shown in Table 2. The overall mean menthol concentration in *zarda PM* samples was significantly ($F1, 616 = 33.58$, $p < 0.001$) higher than *sada* samples. It is interesting to note that significant differences were observed in menthol content in *PM* samples of different zones ($F4, 616 = 14.73$, $p < 0.01$). In hill zone, the mean menthol concentration was not significantly different between *sada* and *zarda PM* samples. However, in the other zones, the differences were significant. The menthol contents in Bhundelkhand and Hill zones were similar and significantly lower than those of other regions. Further, mean menthol content in packed samples were significantly lower than those of non-packed samples in all the zones except West zone, where the trend was slightly reverse.

Among the 50 different brands of *PM* tested in a total of 303 packed samples, 4 brands showed that the menthol contents were within the permissible limits while in 46 brands, these exceeded the prescribed limits (data not shown). These results suggested that good manufacturing practices should be adopted by the industries to prepared *PM* samples, not exceeding the prescribed limit (0.1 %).

Although few reports indicate the toxicity of menthol at high doses but US-FDA and Flavouring Extract Manufacturer's Association (FEMA 1965) have given it GRAS (Generally Recognised As Safe) status. Joint FAO/WHO Expert Committee on Food Aditives (JECFA 1968) has given an unconditional ADI of 0.2 mg/kg for menthol (Opdyke 1976). Based on a survey conducted by ITRC, Lucknow on intake pattern of *PM* in habbitual consumers (ITRC Report, 1993), the Prevention of Food Adulteration Act of India, 1954 has prescribed 0.1 % as maximum level of addition of menthol in *PM* (PFA 1997). From the present study, it was apparent that only 9 % of *PM* samples had menthol within the prescribed limits. The consumption of the remaining 90 % *PM* samples with menthol ranging from 0.11 to 1.0 %, by habitual consumers may develop long term toxic effects which may affect their health.

TABLE 2. COMPARISON OF MENTHOL CONTENT IN *SADA* VS *ZARDA* SAMPLES AND PACKED VS NON-PACKED SAMPLES OF *PAN MASALA* IN DIFFERENT ZONES OF UTTAR PRADESH

Zones		*Sada*	*Zarda*	Total	Packed	Non-packed	Total
East	Number	112	78	190	106	84	190
	SUMSQ	2876.5	2249.8	4326.3	2872.4	1443.9	4316.3
	Mean	3.73	5.03	4.26	4.8	3.47	4.25
	SE	0.20	0.21	0.15	0.18	0.25	0.16
West	Number	123	87	210	113	97	210
	SUMSQ	2200.6	1801.8	4002.4	1837.7	2172.3	4010.0
	Mean	3.48	4.00	3.7	3.0	4.38	3.68
	SE	0.21	0.23	0.16	0.25	0.18	0.16
Bhundel Khand	Number	31	28	51	29	22	51
	SUMSQ	6307.7	519.28	1149.98	763.96	393.93	1157.69
	Mean	0.59	4.84	2.25	4.91	3.79	4.43
	SE	0.82	0.37	0.59	0.28	0.41	0.25
Hill	Number	44	37	81	34	47	81
	SUMSQ	568.8	313.76	882.56	412.98	482.75	895.73
	Mean	2.67	2.47	2.58	3.10	2.22	2.59
	SE	0.36	0.25	0.23	0.28	0.34	0.23
Central	Number	54	36	90	37	53	90
	SUMSQ	970.57	1243.8	2214.37	1386.9	825.83	2212.32
	Mean	3.81	5.57	4.52	5.8	3.62	4.51
	SE	0.25	0.31	0.21	0.28	0.34	0.23
Total	Number	364	258	622	319	303	622
	SUMSQ	6447.17	6128.44	12575.61	7273.94	5318.3	12592.24
	Mean	3.26	4.38	3.73	4.16	3.62	3.90
	SE	0.14	0.13	0.10	0.13	0.12	0.09
Anova	Corr Fact			8629.52			9810.48
Source DF	SUMSQ	MSQ	F Ratio	Source DF	SUMSQ	MSQ	F Ratio
Type 1	187.05	187.05	33.54	Type 1	112.80	112.81	9.69
Zone 4	328.18	82.04	14.73	Zone 4	191.60	47.90	11.73
error 616	3430.86	5.57		error 581	2326.70	4.00	
Total 621	3946.09			Total 586	2631.11		

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RESEARCH PAPER

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Effect of Storage on Physico-chemical and Nutritional Characteristics of Carrot-Beetroot and Carrot-Black Carrot Juices

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Four different combinations of carrot juice with two levels each of beetroot (5 and 10 %) and black carrot (10 and 20 %) juice were prepared and stored for 6 months in glass bottles at room temperature (25° ± 3°C). The juices were tested for sensory scores and physico-chemical and nutritional characteristics every month. Mean sensory scores of juices decreased non-significantly and no significant changes were observed in pH, acidity, total solids, viscosity and mineral contents of the juices during storage. During pasteurization, 11.11-13.09 % ascorbic acid and 14.00-20.47 % β-carotene were lost, while storage for 6 months resulted in 71.26-80.28 % losses in ascorbic acid and 56.60-66.57 % losses in β-carotene contents.

Keywords : Carrot juice, Sensory characteristics, Storage changes, Vitamin C, β-carotene.

Vegetables are important constituents of the diet and provide significant quantities of nutrients especially vitamins, minerals and fibre, depending upon their nature. Green yellow vegetables were originally defined as vegetables containing ≥ 0.6 mg carotene/100 g of edible part. These vegetables reduce the risk of cancer, heart disease, premature aging, stress and fatigue primarily due to integrated action of oxygen radical scavengers such as β-carotene and ascorbic acid plus calcium and dietary fibre (Hirayana 1995). Among the vegetables, carrot (*Daucus carota*) is a rich source of carotene and contains appreciable amounts of B-vitamins, anthocyanin pigments and minerals. Characteristic flavour and aroma of the fresh vegetables are difficult to be retained in processed foods. Vegetables and their juices are subjected to considerable alterations during preparation and processing. Because of the fact that vegetables differ so much in their morphological and physiological characteristics, each presents a unique preparation problem. Carrot has a tremendous potential use as cooked or as a boiled vegetable and in raw form as a part of salad soups and curries. Carrot juice can also be prepared but it is not consumed as such because of its bitter taste and it needs special attention for its processing to develop an acceptable beverage. Processing of carrot juice in India has not received adequate attention, though it is a vegetable of considerable economic importance. Annual carrot production in India is 4 lakh tonnes, while Punjab alone produces 16,000 tonnes, (Anon 1997). Keeping in view the nutritional and therapeutic value of carrots and to make them available throughout the year, carrot juices in combination with other vegetables and fruit juices were prepared, stored and tested for various characteristics.

Materials and Methods

Fresh and disease-free carrots, beetroot, black carrot, lemon and ginger were procured from the local market and washed thoroughly under running water. All vegetables were passed through stainless steel superfine pulper separately to extract juice after peeling. Lemon was cut into halves and squeezed with lemon squeezer. The juices were mixed in 95:5 and 90.10 proportions for carrot : beetroot and 90:10 and 80:20 proportions for carrot : black carrot on v/v basis. To these juices, 5 % lemon and 2 % ginger juices were added. In addition, 6.0 and 6.5 % sugar and 0.24 and 0.25 % black salt were added to improve the taste and flavour of the juices. Sodium benzoate was added at the rate of 140 mg/l of juice. Juice was filled in steriliezed (160°C for 1 h) glass bottles of 200 ml capacity and closed using sterilized crown corks. The filled bottles were pasteurized in hot water at 80-82°C for 25 min and stored for a period of 6 months at room temperature, which ranged from 32 to 40 °C during the storage period.

A trained panel of 10 judges using ranking method according to Larmond (1982) did the organoleptic evaluation. The juices, which were liked the most (excellent) were given a score of 5 and least preferred (poor) were assigned the score of 1. Total solids, titrable acidity, specific gravity and pH of the fresh and stored samples were measured at monthly intervals following AOAC (1985) methods, while viscosity was determined by the method of Roy and Sen (1984). Total bacterial counts of the juices were measured by Pour Plate method as described by Cruickshank (1969). Ascorbic acid and tannins were estimated by colorimetric methods and β-carotene by column chromatography method as described by Ranganna (1995). Total and reducing sugars were estimated following Lane and Eylons method (AOAC 1985). Vegetable juices were directly analyzed on an Inductively Coupled Argon Plasma Emission Spectrophotometer for sodium, potassium, calcium, phosphorus and iron, following the method of Jones (1997). All estimations were carried out in triplicate at monthly intervals except for minerals for which analysis was done after every third month. Results were statistically analyzed using analysis of variance.

Results and Discussion

The data on sensory scores of various carrot juice combinations are presented in Table 1. The results revealed that appearance, colour, flavour, taste and overall accptability of the fresh 4 juices did not differ significantly and ranged between scores as good and very good. Pasteurization and storage of samples did show decrease in various organoleptic

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TABLE 1 SENSORY SCORES OF VARIOUS CARROT JUICE COMBINATIONS (MEAN ± SEM)

Characteristics	Fresh	Pasteurized	Storage period, months 1	2	4	5	F-Ratio
Carrot-Beetroot (95:5)							
Appearance	3.7 ± 0.14	3.6 ± 0.21	3.5 ± 0.21	3.5 ± 0.16	3.4 ± 0.29	3.4 ± 0.21	0.250^{NS}
Colour	3.7 ± 0.14	3.7 ± 0.20	3.5 ± 0.21	3.5 ± 0.16	3.3 ± 0.25	3.3 ± 0.20	0.690^{NS}
Flavour	3.5 ± 0.21	3.5 ± 0.29	3.3 ± 0.32	3.3 ± 0.14	3.1 ± 0.17	3.0 ± 0.20	0.607^{NS}
Taste	3.4 ± 0.21	3.3 ± 0.25	3.1 ± 0.26	3.1 ± 0.22	2.9 ± 0.22	2.9 ± 0.17	0.549^{NS}
Overall acceptability	3.5 ± 0.21	3.3 ± 0.25	3.3 ± 0.28	3.2 ± 0.13	3.1 ± 0.27	3.1 ± 0.17	0.428^{NS}
Carrot-Beetroot (90:10)							
Appearance	3.7 ± 0.25	3.6 ± 0.21	3.6 ± 0.15	3.6 ± 0.21	3.4 ± 0.25	3.4 ± 0.15	0.547^{NS}
Colour	3.7 ± 0.25	3.6 ± 0.21	3.5 ± 0.16	3.5 ± 0.21	3.4 ± 0.21	3.3 ± 0.20	0.354^{NS}
Flavour	3.2 ± 0.24	3.3 ± 0.25	3.0 ± 0.25	3.1 ± 0.22	2.9 ± 0.26	2.9 ± 0.30	0.322^{NS}
Taste	3.3 ± 0.32	3.3 ± 0.28	3.0 ± 0.40	3.0 ± 0.27	2.8 ± 0.24	2.8 ± 0.31	0.595^{NS}
Overall acceptability	3.5 ± 0.25	3.3 ± 0.25	3.1 ± 0.09	3.0 ± 0.22	3.0 ± 0.28	3.0 ± 0.24	0.513^{NS}
Carrot-Black carrot (90:10)							
Appearance	3.2 ± 0.19	3.2 ± 0.24	3.2 ± 0.31	3.1 ± 0.17	3.0 ± 0.32	3.0 ± 0.32	0.102^{NS}
Colour	3.1 ± 0.17	3.2 ± 0.28	3.2 ± 0.28	3.2 ± 0.19	3.0 ± 0.35	3.1 ± 0.30	0.076^{NS}
Flavour	3.2 ± 0.19	3.0 ± 0.25	2.9 ± 0.30	2.8 ± 0.19	2.7 ± 0.25	2.8 ± 0.31	0.052^{NS}
Taste	3.0 ± 0.19	2.9 ± 0.26	2.9 ± 0.30	2.8 ± 0.28	2.9 ± 0.22	2.9 ± 0.22	0.052^{NS}
Overall acceptability	3.4 ± 0.21	3.2 ± 0.19	3.1 ± 0.26	3.0 ± 0.28	3.0 ± 0.20	2.9 ± 0.33	0.406^{NS}
Carrot-Black carrot (80:20)							
Appearance	3.3 ± 0.14	3.1 ± 0.17	3.1 ± 0.26	3.0 ± 0.20	3.1 ± 0.33	3.1 ± 0.30	0.132^{NS}
Colour	3.0 ± 0.15	3.1 ± 0.17	3.0 ± 0.25	3.0 ± 0.25	2.9 ± 0.41	3.0 ± 0.31	0.122^{NS}
Flavour	2.8 ± 0.24	2.8 ± 0.24	2.6 ± 0.21	2.6 ± 0.15	2.5 ± 0.21	2.5 ± 0.38	0.239^{NS}
Taste	2.9 ± 0.22	2.8 ± 0.13	2.7 ± 0.20	2.7 ± 0.25	2.6 ± 0.25	2.6 ± 0.29	0.220^{NS}
Overall acceptability	3.0 ± 0.25	3.0 ± 0.14	2.9 ± 0.22	2.8 ± 0.28	2.7 ± 0.28	2.7 ± 0.40	0.240^{NS}

NS = Non-significant

characteristics but differences were not significant. The flavour, taste and overall acceptability scores were minimum for 80:20 carrot: black carrot combination and maximum for 95:5 carrot: beetroot combination.

The data on various physico-chemical characteristics of various carrot juices are presented in Table 2. The pH of the fresh juices ranged from 3.6 to 3.8, as the juice was intentionally acidified with lemon juice to bring the pH below 4.0 for better keeping quality, taste and flavour. Singh (1990) reported that pH of plain carrot and beetroot juice was 6.3 each, while Singh (1996) reported pH of plain carrot juice ranging from 6.18 to 6.24. Total acidity of the fresh juices ranged from 0.40 to 0.43 %. Sethi (1990) reported acidity of black carrot juice to be 0.32 %, while Khan et al (1988) reported that the acidity of plain carrot juice was 0.16 %, whereas acidity of carrot juice blended with other fruit juices ranged from 0.296 to 0.394 %. Grewal and Jain (1987) reported the acidity of carrot juice beverage to be 0.43 %. Thus, the acidity of the juices is a result of proportions of juices mixed in the final product. Total solids in carrot-black carrot combination were significantly higher ($P<0.05$) than carrot-beetroot combinations. Specific gravity and viscosity of the 4 juices ranged from 1.06 to 1.07 and 80 to 82 sec, respectively and did not differ significantly. Singh (1996) reported that the specific gravity and viscosity of plain carrot juices were 1.060 -1.068 and 26.8 -27.1 sec, respectively. Pasterurization and storage of carrot juices did not affect the pH, acidity, total solids and viscosity of the juices (Bawa and Saini 1987). Total bacterial counts of fresh juices were between 138 x 10^{10} and 141 x 10^{10} cfu/ml, which reduced to negligible level on pasteurization and remained stagnant during the entire storage period. The contamination of juice with bacteria may result from their widespread presence in the air as well as contamination from the extracting machine and utensils used.

Sugars, ascorbic acid, β-carotene and tannin contents of the prepared juices are presented in Table 3. Total sugar contents of various fresh juices ranged from 6.94 to 7.46 % while reducing and non-reducing sugars ranged from 2.52-3.40 and 3.59 - 4.31 %, respectively. Ascorbic acid and β-carotene contents of various juices were 8.1-9.9 and 3.56-4.25 mg/100 ml, respectively. Tannin contents of carrot-black carrot (20 - 21 mg/100 ml) juices were significantly higher ($P<0.05$) than carrot-beetroot juices (14 - 15 mg/100 ml). Pasteurization and storage for 6 months did not affect total sugars and increase in reducing sugars was significant. However, non-reducing sugars decreased significantly ($P<0.05$) after 3 months of storage probably due to slow hydrolysis of sucrose as shown by concomitant reduction in total sugars. Pasterization of juices resulted in 12 % loss of ascorbic acid and 19 % loss of β-carotene and had no affect on tannin content. Storage of juices resulted in significant ($P<0.01$) losses of ascorbic acid and β-carotene specially after 3 months. By the end of 6 months of storage period, 74.7 - 83.9 and 52.0 - 68.3 % of ascorbic acids and β-carotene, respectively

TABLE 2. EFFECT OF STORAGE PERIOD ON PHYSICO-CHEMICAL CHARACTERISTICS OF VARIOUS JUICE COMBINATIONS

	Storage period, months								
	Fresh	Pasteurized	1	2	3	4	5	6	F-Ratio
Carrot-Beetroot (95:5)									
pH	3.80	3.90	4.00	3.80	3.80	3.80	3.80	3.90	2.470^{NS}
Total acidity	0.42	0.42	0.41	0.40	0.41	0.41	0.41	0.41	0.410^{NS}
Total solids	11.56	11.55	11.56	11.56	11.52	11.53	11.52	11.53	3.430^{NS}
Specific gravity	1.06	1.06	1.05	1.06	1.05	1.05	1.06	1.05	0.286^{NS}
Viscosity (seconds)	80	80	80	80	81	81	81	81	0.286^{NS}
Bacterial count (cfu/ml)	138 ± 10^{10}	1	1	2	1	2	2	1	-
Carrot-Beetroot (90:10)									
pH	3.60	3.60	3.70	3.60	3.70	3.70	3.70	3.70	1.330^{NS}
Total acidity	0.43	0.43	0.42	0.41	0.42	0.42	0.43	0.43	0.554^{NS}
Total solids	11.80	11.80	11.79	11.79	11.79	11.77	11.77	11.77	1.710^{NS}
Specific gravity	1.06	1.06	1.05	1.06	1.05	1.06	1.06	1.05	0.268^{NS}
Viscosity (seconds)	81	81	82	82	82	82	82	82	0.214^{NS}
Bacterial count (cfu/ml)	140 ± 10^{10}	2	3	2	2	1	2	2	-
Carrot-Black carrot (90:10)									
pH	3.70	3.70	3.80	3.70	3.70	3.80	3.70	3.70	1.000^{NS}
Total acidity	0.41	0.41	0.41	0.42	0.42	0.42	0.43	0.42	0.219^{NS}
Total solids	13.74	13.74	13.73	13.73	13.73	13.73	13.70	13.70	2.570^{NS}
Specific gravity	1.06	1.06	1.05	1.06	1.05	1.06	1.06	1.05	0.268^{NS}
Viscosity (seconds)	82	82	82	82	82	83	83	83	0.268^{NS}
Bacterial count (cfu/ml)	141 ± 10^{10}	1	2	1	2	3	1	2	-
Carrot-Black carrot (80:20)									
pH	3.80	3.80	3.80	3.70	3.70	3.80	3.80	3.80	1.330^{NS}
Total acidity	0.40	0.41	0.41	0.40	0.41	0.42	0.42	0.42	0.696^{NS}
Total solids	13.88	13.87	13.87	13.87	13.87	13.85	13.84	13.84	2.430^{NS}
Specific gravity	1.07	1.06	1.06	1.05	1.05	1.05	1.06	1.05	0.554^{NS}
Viscosity (seconds)	82	82	83	83	83	83	83	83	0.263^{NS}
Bacterial count (cfu/ml)	140 ± 10^{10}	2	2	1	2	1	3	1	-

NS = Non-significant

TABLE 3. EFFECT OF STORAGE PERIOD ON NUTRIENTS AND TANNINS OF VARIOUS JUICE COMBINATIONS

	Storage period, months									CD at
	Fresh	Pasteurized	1	2	3	4	5	6	F-Ratio	5% level
Carrot-Beetroot (95:5)										
Total sugars, %	6.94	6.94	6.93	6.93	6.92	6.91	6.91	6.90	2.21^{NS}	-
Reducing sugars, %	3.16	3.15	3.16	3.16	3.17	3.19	3.20	3.19	3.35^{NS}	-
Non-reducing sugars, %	3.59	3.6	3.58	3.58	3.56	3.53	3.52	3.52	10.57*	0.048
Ascorbic acid, mg/ 100 ml	8.40	7.30	6.40	5.30	4.0	3.20	2.90	1.60	557.27**	0.475
β-carotene, mg/ 100 ml	3.56	2.88	2.38	2.25	1.75	1.44	1.38	1.25	6626.98**	4.750
Tannins, mg/ 100 ml	14	14	15	16	17	21	22	23	13.64**	4.750
Carrot-Beetroot (90:10)										
Total sugars, %	7.06	7.06	7.06	7.05	7.05	7.04	7.04	7.03	1.27^{NS}	-
Reducing sugars, %	2.52	2.52	2.53	2.54	2.54	2.55	2.53	2.57	2.85^{NS}	-
Non-reducing sugars, %	4.31	4.31	4.30	4.28	4.28	4.27	4.27	4.24	5.64*	0.048
Ascorbic acid, mg/ 100 ml	8.10	7.10	6.30	5.10	3.70	3.00	2.20	1.40	585.84**	0.475
β-carotene, mg/ 100 ml	4.12	3.38	3.00	2.69	2.38	2.25	1.69	1.44	7755.2**	4.750
Tannins, mg/ 100 ml	15	15	15	16	16	18	21	22	7.93**	4.750
Carrot-Black carrot (90:10)										
Total sugars, %	7.46	7.45	7.45	7.45	7.44	7.44	7.43	7.43	1.13^{NS}	-
Reducing sugars, %	3.40	3.41	3.41	3.42	3.42	3.43	3.44	3.44	2.29^{NS}	-
Non-reducing sugars, %	3.86	3.84	3.84	3.84	3.82	3.81	3.79	3.79	6.55*	0.048
Ascorbic acid, mg/100 ml	9.50	8.40	7.0	6.40	5.20	4.50	3.20	2.30	618.70**	0.475
β-carotene, mg/100 ml	4.00	3.44	3.00	2.81	2.56	2.25	1.88	1.38	7095.43**	4.750
Tannins, mg/100 ml	20	20	20	21	21	26	26	27	9.70**	4.750
Carrot-Beetroot (80:10)										
Total sugars, %	7.25	7.25	7.25	7.24	7.24	7.23	7.23	7.22	1.27^{NS}	-
Reducing sugars, %	3.26	3.26	3.26	3.27	3.27	3.28	3.30	3.30	2.85^{NS}	-
Non-reducing sugars, %	3.79	3.79	3.79	3.77	3.77	3.75	3.73	3.72	7.70*	0.048
Ascorbic acid, mg/100 ml	9.90	8.70	7.60	6.00	5.10	4.90	3.80	2.50	630.55**	0.475
β-carotene, mg/100 ml	4.25	3.38	2.25	2.13	2.00	1.69	1.38	1.13	10961.70**	4.750
Tannins, mg/100 ml	-	22	22	22	24	27	27	27	6.86**	4.750

NS = Non significant *Significant at 5 % level, **Significant at 1 % level

TABLE 4 EFFECT OF STORAGE PERIOD ON MINERAL CONTENTS OF VARIOUS CARROT JUICE COMBINATIONS (mg/l)

	Fresh	Pasteurized	Storage period, months 3	6	F-Ratio	CD at 5% level
Carrot-Beetroot (95:5)						
Iron	3.76	3.76	3.78	3.76	1.0^{NS}	-
Sodium	2250	2250	2240	2220	2.00^{NS}	-
Potassium	1910	1900	1890	1880	1.67^{NS}	-
Calcium	78	77	75	50	179.33**	6.5
Phosphorus	163	163	163	161	1.00^{NS}	-
Carrot-Beetroot (90:10)						
Iron	3.87	3.87	3.86	3.88	4.0^{NS}	-
Sodium	1940	1930	1920	1900	2.93^{NS}	-
Potassium	1680	1670	1650	1640	3.33^{NS}	-
Calcium	77	76	74	40	319.58**	6.5
Phosphorus	166	166	165	162	3.58^{NS}	-
Carrot-Black carrot (90:10)						
Iron	4.50	4.50	4.51	4.40	0.667^{NS}	-
Sodium	1880	1880	1860	1850	2.25^{NS}	-
Potassium	2910	2900	2880	2860	4.92^{NS}	-
Calcium	107	107	105	97	22.67**	6.5
Phosphorus	253	253	250	252	2.00^{NS}	-
Carrot-Beetroot (80:20)						
Iron	4.82	4.82	4.80	4.78	3.67^{NS}	-
Sodium	1890	1880	1860	1840	4.92^{NS}	-
Potassium	2910	2910	2900	2880	2.00^{NS}	-
Calcium	113	112	110	96	68.73**	6.5
Phosphorus	268	268	265	264	4.25^{NS}	-

NS = Non-significant **Significant at 1% level

were lost. Losses of ascorbic acid and β-carotene during cooking and storage of tomato, amla and carrots have been reported by various workers (Kwasneiwska et al. 1987; Aggarwl et al. 1995; Dietz and Gould 1986; Nagra and Khan 1988). Pasteurization of the juices did not affect tannic acid but it increased significantly after 3-4 months of storage. Similar increases in tannic acid content of amla juice (Tripathi et al. 1988) and carrot juice (Singh 1990) have been reported during storage for more than 4 months.

Iron, sodium, potassium, calcium and phosphorus contents of various fresh and stored juices (Table 4) indicated that pasteurization of the juices had no effect on any of the minerals. Iron, potassium, calcium and phosphorus of both carrot-black carrot juice were significantly higher than carrot-beetroot juices. Storage of the juices did not affect the mineral contents of the juices except for calcium, which was not affected upto 3 months but between the third and 6th month, a significant ($p<0.01$) reduction was observed. This might be due to binding of calcium with certain organic substances as reported by Gopalan et al (1993).

On the basis of the results of this study, it may be concluded that formulation of mixed vegetable juice beverages is possible to satisfy consumer taste and preferences. These juices can be stored effectively for a period of 3 months without any change in pH acidity, total solids, specific gravity, viscosity, total bacterial counts, sugars, tannins and minerals like sodium, potassium, calcium, phosphorus and iron. Intake of about 100 ml of fresh juice would help to meet the daily requirements of β-carotene and almost one-fourth of the requirements of ascorbic acid.

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RESEARCH PAPER

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Production of Zinc-Enriched Yeast Extracts

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Dried zinc-containing yeasts (5 g zinc / 100 g yeast) were rehydrated at 20 % solids and subjected to autolysis at 50°C for 24 h. Either of the following enzymes, glucoamylase, mannanase, β-1, 3 glucanase, fungal acid protease, validase FP or papain was added to promote lysis. Autolysis yields, recovery of zinc in the yeast extract (YE) as well as composition of the YE (nitrogen, sugars) were determined. In the absence of added enzymes, autolysis yield with fresh Bakers' yeast was 46 %, while that with the rehydrated zinc-enriched yeasts was 21 %. All adjunct enzymes increased autolysis yields (up to 41 %) from the rehydrated zinc-enriched yeast. The fraction of the zinc that was recovered in the YE ranged from 64 to 81 % of the total amount originally present in the yeast, depending on the enzymes used (β-1, 3 glucanase and validase FP gave best results). Yeast extracts obtained from glucanase had higher contents of glucose, but lower contents of α-amino nitrogen than those produced with the proteases. The use of enzymes to obtain YE from rehydrated zinc-containing yeasts generated YE having 15 g of zinc/100 g of yeast extract.

Keywords : Zinc-enriched yeast extracts, Turbidity, Filtration, Amino acids, *Saccharomyces cerevisiae*.

Zinc is known to have many important biochemical functions in human metabolism, mainly due to its role in enzyme activity. There are more than 300 enzymes, requiring zinc for their activities (Vallee and Flachuk 1981; Eide 1998). Therefore, it is essential that diet should contain zinc in adequate quantities to meet the daily requirements (the recommended daily allowance is 15 mg) and accordingly, many commercial vitamin-based supplements include zinc in their formulations.

Some inorganic salts are not as readily available or biologically active as organic forms of minerals (Schwartz 1976; Hegoczki et al. 1997). Mineral-enriched yeasts contain organically bound minerals (Hegoczki et al. 1997) and some products, such as selenium-rich yeast are very effective in promoting health and even reducing the risk of cancer (Clark et al. 1996). Therefore, there is interest in preparing organic sources of minerals.

Saccharomyces cerevisiae is effective in binding zinc (Volesky and May-Phillips 1995), and zinc-enriched yeasts are available in the market. Mineral-enriched yeasts can be used as supplements or as ingredients in speciality foods, such as nutritive bars, but not in many beverages, as they become turbid or deposits are formed. One way to obtain a soluble form of yeast is to produce yeast extracts (YE), since yeasts whose cell wall has been destroyed, can be added to foods and beverages, for example juices (Oriental Yeast Co, Ltd 1996). Yeast extracts (YE) are concentrates of soluble material obtained from yeast, following autolysis in controlled conditions (Kelly 1973). They can be used as ingredients of foodstuffs, feeds and fermentation media (Dziedzak 1987). The nutritive value of YE is related to its high content of soluble nitrogen as well as B-vitamins, phosphorus and trace elements (Peppler 1982; Reed and Nagodawithana 1991). Yeast extracts could then be a useful and valuable means of adding zinc to foods and beverages meant for human consumption. However, information is scanty on the production of zinc-enriched YE by lysis of the yeast.

The aim of this study was, therefore, to determine the influence of enzymes on the production of zinc-enriched yeast extracts from dried mineral-enriched Bakers' yeast and to evaluate some of their characteristics.

Materials and Methods

Yeasts : Dried inactive zinc-enriched yeasts (50 g/kg of zinc) were kindly provided by Lallemand Inc. Montreal, Canada. A 20 % solid (w/w) yeast suspension was prepared by rehydrating in deionized water. For one series of assays, fresh (compressed) Bakers' yeast was used.

Enzymes : Glucoamylase, mannanase, β-1, 3 glucanase and fungal acid protease were from Enzyme Development Corporation (EDC, New York, USA), while validase FP concentrate and papain were obtained from Valley Research Inc. South Bend, USA.

Yeasts cell enzymatic digestion : The commercial enzymes were added to the yeast suspensions at 0.1% w/w of total yeast solids and the mixture was incubated at 50°C for 24 h. The pH was 4.9 ± 0.2 during the autolysis period. Following this incubation, the lysed yeasts were centrifuged at 15000 g for 15 min. The pellet was washed with deionized water, vigorously stirred and centrifuged again for 15 min. The supernatants from both centrifugations were pooled, the volume determined and samples were taken for determination of total solids and chemical composition. The YEs were placed in freeze-drying pans in order to obtain 1 cm thick layers and frozen at -40°C. They were then freeze-dried in a Lyo-Tech (LYO-SAN Inc., Lachute, Canada) unit at 24°C for 48 h, under a vacuum of at least 100 μ. Four independent assays were done.

Determination of total solids : Total solid contents of all extracts were obtained by dry weights after drying at 105°C for 16 h.

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Zinc concentration determination : Crucibles were washed before hand in 10 % HCl for 2 h, rinsed with deionized water and dried in an oven at 550°C for 1 h. They were removed from the oven and placed in a desiccator for 30 min. Samples (2 g) were added to the cooled crucibles. They were dried at 105°C and then ashed at 550°C for at least 16 h. The samples were cooled in a desiccator for 30 min and weighed when they reached the room temperature. The ashed content was diluted in 10 ml concentrated HCl and evaporated to dryness on hot plates. The residue was dissolved again by adding 10 ml concentrated HCl. Zinc concentration was determined on this solution by atomic absorption with a Varian Spectr AA-100 (Malgruve, Victoria, Australia). A specific hollow cathode lamp (model 3QNYZn-SH, Cathodeon Limited, Cambridge, UK) and 213.9 nm wavelength were used to determine zinc concentrations in extracts.

Determination of α-amino nitrogen of yeast extracts : The α-amino nitrogen of the fractions was determined by titration, following reaction with formaldehyde (USP 1985). The 5% YE solutions were adjusted to pH 7.0 with 0.1 M NaOH or 0.1 M HCl.

Determination of total nitrogen in yeast extracts : The total nitrogen content was determined, using a FP-428 LECO apparatus (LECO Corporation, Saint Joseph, MI), operated under the following conditions : sample size, 150 mg ; oxidation furnace temperature, 900°C ; oxidation standby temperature, 650°C ; purge cycles, 3 ; minimum timeout, 30 ; comparator level, 1.00; loop select "Low" range, Flow constants: "High", gases : oxygen 99.99% and helium 99.99 %. The calibration standard was composed of 150 mg EDTA (n° 502-092, 9.56 ± 0.03 % Nitrogen, LECO Corporation, Saint Joseph, MI).

Sugar analyses of yeast etracts : Sugars (glucose, mannose and trehalose) were determined by HPLC with the method developed by Doyon et al (1991), using a cation exchange column Aminex HPX 87H (300 X 7.8 mm; BioRad, Mississauga, Canada). The elution fluid was 0.008 N H_2SO_4 at 40°C and the analysis was done on a Waters (Cortland NY. USA) system composed of the following components : model 717 autosamples, 510 pump and 410 differential refractometer. The HPLC method used to quantify the sugar contents of the extracts could not separate the standards, trehalose and sucrose. For this reason, the term trehalose-equavalent was used.

Statistical analysis : Four different lots of zinc-enriched yeast were used and results were the average of four independent trials. Stastical analyses were conducted using SAS software (SAS Institute, Cary, NC). Analysis of variance was used with significance defined at $P \leq 0.05$.

Results and Discussion

Autolysis yields : Autolysis yield with fresh Bakers' yeast was 46%, which was in line with that reported by Kelly (1973). Results with the inactive dry zinc-enriched yeasts were thus low at 21.3 %(Table 1). This suggested that the native yeast enzymes were not very active during lysis of yeast cells. This could be related to enzyme denaturation during drying. Adjunct enzymes were consequently added to yeast cell suspensions to enhance enzymatic lysis of yeast components. The enzymes were added on a weight basis (0.1 %) of the commercial preparation rather than on an activity basis. This approach was used since no comparative activity data (same substrate and conditions) between these enzymes were available and that the enzymes were designed to act on different portions of the yeast cell. The fact that different quantities of enzymes may have been used must be kept in mind in the interpretation of the results.

All enzymes increased yields in comparison with the control, β-1, 3 glucanase and validase FP being the most effective (Table 1). The major function of β-1, 3 glucanase enzyme is to dissolve the yeast cell wall (Cenamor et al. 1987). This particular enzyme mixture contains mainly β-1,3 glucanase, in addition, it also contains amylase, β-glucosidase and protease (EDC, Technical Sheet). The validase FP mixture rather contains exo-and endo-protease (Valley Research, Technical Sheet). Therefore, both glucanases and proteases were effective in increasing the solubilization of the yeast. It was not determined by combining the enzymes or modifying the incubation conditions (temperature, pH) to better suit the particular enzyme's optimum conditions and further increase yields. The literature suggests this could be the case (Kelly 1973; Ryan and Ward 1985 , 1988) and further studies in this respect are warranted.

Higher lysis yields were expected with papain, since this enzyme is recognized as being very effective for this purpose (Kelly 1973; Hobson and Anderson 1991). Zinc is known to inhibit the activity of proteases (Rattray et al. 1995) and other enzymes (Kadowaki et al. 1996; Munshi et al. 1993; Nadachi and Hirota 1992; Rollan et al. 1989), but no data are available on the effect of zinc at so high levels on the activity of papain. Data obtained from the present study suggest that some inhibition occurs.

Zinc recovery : Although only 21.3 % of the initial solids in the dried yeast were solubilized during the autolysis process, 64.5 % of the total zinc was recovered. Therefore, the YE contained 15 g of zinc/100g of YE. The YE contained on the average 4.5% humidity after freeze-drying.

All enzymes increased recovery of zinc in the YE (Table 1) in comparison with the amount of soluble zinc in the control (64.5 %). This suggested that a correlation between YE yields and zinc solubilization existed and regression analyses were thus conducted. The first order relationship between YE yields and zinc recovery in the YE gave an R^2 of 0.72, which showed a significant correlation, although it was not very high. As was the case for yields, β-1, 3 glucanase and validase FP enzyme preparations gave the highest results (Table 1). It is noteworthy that zinc is liberated, following the activity of both proteases and glucanases. Genetic analyses suggest that almost 3% of *S. cerevisiae* proteins potentially contain zinc-binding domains (Eide 1998), which would explain the important level of zinc release following proteolysis.

Cation uptake by yeasts is typically biphasic, involving rapid metabolism-independent binding to cell surfaces, followed by a slower phase of metabolism-dependent intracellular uptake

TABLE 1. EFFECT OF ENZYMES ON YIELDS AND ZINC SOLUBILIZATION FOLLOWING A 24 H AUTOLYSIS OF ZINC-ENRICHED YEASTS (THE INITIAL Zn CONTENT WAS 5g/100 g DRIED YEAST)

Enzyme	Yeast extract yield, %	Total Zinc recovered, %
Control (no enzyme added)	21.3^{c}	64.5^{b}
Papain	26.0^{bc}	72.8^{ab}
Fungal acid protease	28.4^{bc}	79.0^{ab}
Mannanase	28.8^{bc}	76.3^{ab}
Glucoamylase	29.2^{bc}	75.8^{ab}
β-1, 3 glucanase	31.9^{ab}	80.0^{a}
Validase FP	40.7^{a}	81.8^{a}

abc : For a given column, values that are followed by the same letter are not considered to be significantly different (P>0.05)

TABLE 2. NITROGEN AND SUGAR CONTENTS OF ZINC-ENRICHED YEAST EXTRACTS. VALUES ARE IN g/100 g OF YEAST EXTRACT SOLIDS

Enzyme	α-amino nitrogen	Glucose	Trehalose-equivalent
Control (no enzyme added)	5.7^{c}	0^{c}	0.84^{ab}
Papain	5.5^{a}	0^{c}	0.82^{ab}
Fungal acid protease	5.1^{a}	0.40^{bc}	0.76^{c}
Mannanase	5.5^{ab}	0.80^{ab}	0.80^{bc}
Glucoamylase	5.5^{ab}	1.20^{a}	0.85^{a}
β-1, 3 glucanase	4.6^{b}	0.40^{bc}	0.70^{d}
Validase FP	5.3^{ab}	0.06^{c}	0.64^{c}

abc : For a given column, values that are followed by the same letter are not considered to be significantly different (P>0.05)

(Failla et al. 1976 ; Gadd and Mowll 1983 ; Mowll and Gadd 1983). The data from this study suggested that a significant fraction of zinc (up to 17 %) was internally located. *Saccharomyces cerevisiae* has multiple zinc transporters that enable the absorption of the metal (Radisky and Kaplan 1999). Furthermore, the significant intracellular zinc content could be associated wit the presence of intracellular vesicular compartments that contribute in detoxifying excess zinc (Eide 1998). The present finding is in line with that of Volesky and May-Phillips (1995), who showed that *Saccharomyces cerevisiae* was more effective in adsorbing zinc rather than absorbing it. It remains to be determined what proportion of the 64 % of zinc that were released in the control assays was free or loosely bound to the cell surface.

Nitrogen content of enriched yeast extracts : In autolysis assays conducted with fresh (compressed) Bakers' yeast, the total nitrogen content of the YE was typically between 8 and 11 g/100 g of solids, while that of α-amino nitrogen varied between 3 and 7 g/100g. The α-amino nitrogen data obtained with the zinc-enriched products were in line with these results (Table 2). However, the methodology used in this study for the determination of total nitrogen of YE gave values between 3.5 and 5.5 g/100g of YE, which was clearly an under-estimation of the total nitrogen content of the YE.

The addition of various enzymes to carry out yeast autolysis had no significant effect on the proportional α-amino nitrogen content of the extracts produced except for β-1, 3 glucanase (Table 2). The lower α-amino nitrogen content of the YE obtained with β-1, 3 glucanase is logical, in view of the fact that this enzyme preparation does not mainly contribute to protein hydrolysis.

Sugar content of the yeast extracts : The sugar contents of the zinc-enriched YE produced with various enzymes are shown in Table 2. The extracts produced with the proteases had the lowest glucose values. No mannose was found in the extracts. However, the trehalose-equivalent content was very much different within the extracts and the lowest amount was found in the validase FP-produced extract.

Conclusion

The addition of adjunct enzymes to inactive dry zinc-enriched yeast creams allowed the production of YE containing up to 15 % zinc. The study showed that a significant portion of the zinc in yeast could only be released through enzyme action, which suggested a binding to cell structures or that the zinc was located inside the cell. These zinc-containing YE could be added to human foods and beverages. However, the form of the zinc in the YE remains to be determined, which is a critical feature of the organically-bound mineral supplements market. Furthermore, sensory analyses will need to be conducted to determine the effect of enzyme treatment of the flavour of the YE.

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RESEARCH PAPER

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Osmotic Dehydration Characteristics of Button Mushrooms

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Osmotic drying behaviour of button mushrooms was experimentally studied in relation to temperature (25, 40 and 55°C), solution to sample ratio (4, 6 and 8) at a fixed brine solution concentration of 15 %. Osmosis could remove almost 35 % of the initial moisture in one hour, using 15 % brine solution. The modelling of moisture loss, salt gain and mushroom solids loss revealed that out of power law, log, generalized exponential and Page's model, Page's model could describe the moisture loss pattern most accurately whereas, salt gain and solid loss could be best described by power law and log models, respectively.

Keywords : Osmosis, Button mushroom (*Agaricus bisporus*), Temperature, Solution to sample ratio, Moisture loss, Salt content.

Osmotic processing has attracted considerable research interest, due to its potential to partially desiccate high moisture foods, maintain product quality and stability, modify functional properties, consequently resulting in substantial energy savings (Lerici et al. 1988; Nanjundaswamy and Radhakrishnaiah Shetty 1989). The benefits and drawbacks of osmotic dehydration have been extensively reviewed (Roult-Wach et al. 1992 ; Torreggiani 1993 ; Lazarides 1993).

Despite the recognized advantages of the osmotic treatment, its commercial applications are still quite limited. Problems associated with management and microbial stability of the osmotic solution coupled with difficulties to control undesirable solid uptake are probably the main reasons for its slow industrial development (Roult-Wack 1994; Lazarides et al. 1995).

The influence of main process variables e.g., pre-treatment applied to feed, the type of osmotic agent, the concentration and composition of osmotic solution, temperature, process time, agitation, solution to sample ratio etc. on the mass transfer mechanism and the product quality has been studied extensively (Lenart and Lewiki 1990 ; Yang and Le Maguer 1992 ; Shi and Maupoey 1993 ; Grabowski et al. 1994 ; Alvarez et al. 1995 ; Lazarides et al. 1995 ; Vijayanand et al. 1995 ; Rastogi et al. 1997 ; Yao and Le Maguer 1997).

During osmotic pre-concentration, two major concurrent flows take place simultaneously under the water and solute activity gradients across the semi-permeable cell membrane, water flows from the product into the osmotic medium, while osmotic solute is transferred from the medium into the products (Lenart and Flink 1984). A third transfer process, leaching of product solutes (sugar, acids, minerals and vitamins) into the medium, although recognized as affecting the organoleptic and nutritional characteristics of the products, is considered quantitatively negligible (Dixon and Jen 1977).

Both solid gain and water loss increase with the increase of syrup and food mass ratio. Lenart and Flink (1984) reported that, at equilibrium, the solids gain content was approximately 75 % at a solution to potato ratio of 2, which showed only a small liner increase (about 3%) over a rated range of ratios of 2-10 and considering water activity, solids gain and economics, they recommended a ratio of 4. Uddin and Islam (1985) studied the effect of syrup to pineapple slice mass ratio on osmosis of pineapple at 21°C. The observed increased weight loss up to a ratio of 4:1, but by increasing the ratio further upto 6:1, no such gain was observed. Thus, they defined an optimum ratio for pineapple as 4:1. Drying rates have been reported to increase with increase in osmotic syrup concentration (Biswal et al. 1991 ; Lazarides et al. 1995 ; Vijayanand et al. 1995).

Yang and Le Maguer (1992) conducted osmotic dehydration of bottom mushroom (*Agaricus bisporus*) in a continuously circulated contacting reactor. The treatments studied were (i) Sliced mushrooms were blanched and osmotically processed in 5, 10 and 15 % salt solutions for 5, 10, 20 and 30 min. at room temperature; and (ii) Sliced and blanched mushrooms were osmotically dehydrated in 60 % sucrose solution for 10 min at 50°C and then transferred to the same processing conditions as mentioned. They concluded that 15 % NaCl solution was optimum for both types of processing conditions. Pre-treatment of mushrooms in high concentration of sucrose solution, followed by treatment in high salt concentration was the most effective method to remove water and loading salt to further lower the water activity in the mushroom.

Inadequate control of product characteristics such as raw material variability in terms of variety, maturity level and even cultivation procedures drastically affect the composition and barrier properties of a specific fruit/vegetable and the complex non-homogenous structure of natural tissues seriously complicates the modelling of mass transport mechanisms of several interacting counter current flows (water, osmotic solute, soluble product solids). Many empirical, semi-empirical and theoretical models have been developed for osmotic dehydration of fruits and vegetables, taking into account the outgoing water, incoming solute, shrinkage of fruit during the process, transmembrane flux, bulk flow velocity, etc. (Hough

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et al. 1993; Rastogi and Raghavarao 1995; Rastogi et al. 1997; Yao and Le Maguer 1997). However, in the present study, only simple empirical models were tried.

The present study was undertaken to study the effect of temperature and solution to sample ratio on osmotic drying characteristics of mushroom quarters, optimize the osmotic conditions, based upon minimum solids gain and maximum water loss and model the kinetics of moisture loss, salt gain and mushroom solids loss.

Materials and Methods

Compact and fresh mushrooms (*Agaricus bisporous*) procured from the Mushroom Research Centre of the G.B. Pant University of Agriculture and Technology were used in the study. Each mushroom was cut into quarters and thoroughly washed under tap water to remove adhering impurities. It was subsequently washed in 0.1 % KMS solution (approx. 3 times volume per unit weight for 5 min.) in order to remove any remaining impurities.

The brine solution was prepared by dissolving the required quantity of NaCl (w/v) in distilled water.

Preliminary experiments : Initially, osmotic dehydration experiments were carried out for about 20 h to study the kinetics of moisture loss and salt gain. There was significant loss of moisture in the first hour after which salt gain dominated the process. Therefore, it was decided to carry out osmotic dehydration for one hour.

Experimental procedure : Mushrooms cut to quarters were subjected to osmosis in a beaker, containing salt solution of the desired concentration of 15 % and 4, 6 and 8 times the volume per unit weight of the sample. Adequate arrangement was made to ensure complete submergence of the mushroom quarters in the salt solution. The temperature was maintained by a thermostatically controlled water bath. Temperature variation was not more than ± 1°C. The mushrooms were taken out of beaker after 5, 10, 15, 20, 30, 35 and 60 min of osmosis. The material was drained on a sieve (8 holes per linear inch) for 10 min to remove the adhering brine. It was subsequently put on a blotting paper for one minute to ensure complete removal of surface moisture, if any, retained after sieving. Initial and final weights, moisture and salt contents of the mushroom quarters subjected to osmosis after each time interval were recorded. Moisture content was determined by hot air oven method and salt analysis by direct titration method of Mohr (Ranganna 1986).

Results and Discussion

The initial moisture content of mushrooms varied from 89.9 to 92.5 % (w.b). The moisture loss, salt gain and non-salt solid loss were 35 %, 75 % and 25 %, respectively during one hour osmosis for the entire range of experimental variable. These changes were significantly different between temperatures of 25 and 40°C, but were insignificant for the temperatures of 40°C and 55°C. The solution to sample ratio however, had an insignificant effect on moisture loss whereas, it significantly contributed to the salt gain and non-salt solid loss between solution to sample ratio of 4 and 6.

Moisture profile : In view of initial variation in moisture content, the moisture profile was analysed in terms of ratio of mass of moisture present after osmosis to the initial mass of moisture. The rate of moisture loss from mushroom was faster at higher temperature, since at a solution to sample ratio of 4, 27,9 % of initial moisture was removed at 25°C in one hour corresponding to 39.2 % at 55°C (Table 1). Similarly, moisture loss ratio also increased with increase in solution to sample ratio at a temperature of 95°C but at 40 and 55°C, the difference in moisture levels was small being in the range of 38.70 - 39.45 % as compared to 27.90 - 30.96 % present at 25°C. In view of significant difference in the moisture removal rate between 25 and 40°C and insignificant effect between 40 and 55°C, 40°C was taken as the optimum temperature for osmosis. Similarly, as effect of solution to sample ratio was statistically insignificant on the moisture loss rates, and at 40°C, dry basis moisture ratio was the lowest at solution to sample ratio of 6, indicating maximum loss of moisture, this ratio of 6 was accepted. The loss of moisture from the mushroom non-linearly increased with time of osmosis, for all conditions (Fig. 1). In general, the moisture loss was faster in the initial stages of osmosis and subsequently decreased, owing to decrease in moisture difference between the product and the solution, resulting in a lower driving force. Moreover, choking of some of the pore spaces of the mushroom due to entry of the salt into the body of the mushroom may also be the cause of lower moisture loss rate kinetics due to hindrances in the movement of water from the centre to the

TABLE 1. OSMOSIS CHARACTERISTICS AT THE END OF ONE HOUR

Temperature, °C	Solution to sample ratio								
	4			6			8		
	ML	SC	MSL	ML	SC	MSL	ML	SC	MSL
25	27.90	33.68	15.35	30.00	43.37	25.38	30.96	43.91	29.56
40	38.70	41.29	23.16	38.97	48.26	28.25	38.94	48.81	33.80
55	39.20	47.47	30.28	39.25	52.34	39.18	39.45	52.68	39.50

ML = moisture loss (% of initial moisture)
SC = salt content (% of initial dry matter)
MSL = mushroom solid loss (% of initial dry matter)

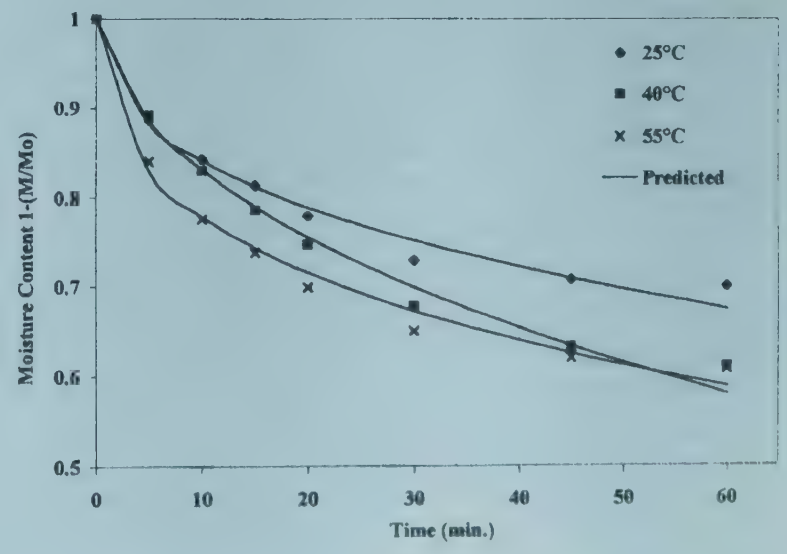

Fig. 1. Performance of Page's model in predicting moisture content at solution to sample ratio 6

surface of the mushroom. For example, at 25°C and solution to sample ratio of 4, the rate of moisture removal was 1.382 % of initial moisture per minute in the first 5 min, which decreased to nearly half, i.e., 0.784 g/g in the next 5 min and to about 1/10th of the initial rate during the last 5 min. Similar general pattern was observed at all other conditions. The validity of the following models was tested through regression analysis.

Power law : $M/M_o = K t^b$ ..... (1)

Log model : $M/M_o = a+b \log t$ ..... (2)

Generalized exponential model : $(1-M/M_o) = a e^{-kt}$ ..... (3)

Page's model : $(1-M/M_o) = \exp(kt^{-kt})$ ..... (4)

where

k, a, b and n = constants

$(1-M/M_o)$ = Moisture loss ratio

t = Time period, min

The models were fitted to the experimental data separately for each condition of osmosis, using lotus package on Compaq Prolinea 450. In general, all the models represented the pattern of moisture change reasonably well, as the values of r^2 ranged from 0.900 to 0.999 and the standard error varied from 0.010 to 2.250. In order to select the best fitting model, in addition to r^2 and standard error, prediction error for each observation was calculated. On the basis of these three parameters, it was found that Page's model could describe the result most precisely, since the moisture content at any time could be predicted with a maximum deviation of 0.25 %.

As no relationship between the model constants obtained using the above equations and osmosis conditions could be established, individual values of constants were required for predicting the moisture loss behaviour of mushroom during osmosis.

In order to develop a general model relating moisture loss with various process parameters, an analysis of lumped data for the 63 sets were carried out, using power law and log models. Although the values of r^2 were more than 0.9 in both the cases, the value of r^2 was slightly higher in case of log model and standard error was considerably smaller. On the basis of this analysis, the log model was considered to best describe the moisture change. The final form of the model was :

$M/M_o = a + b\log t + c\log T + d\log R$ (r^2 = 0.954) ..... (5)

with values of a, b, c and d being -0.507, 0.229, 0.252 and 0.976, respectively. These values indicated that temperature was more predominant in comparison to the solution to sample ratio.

Salt content : The fresh mushroom, prior to osmosis, did not have any salt. However, the salt content of mushrooms increased rapidly with the time of osmosis. The salt content was expressed as % of initial dry matter. The salt content varied from 33.68 % at a temperature of 25°C and solution to sample ratio of 4 to 52.68 % at a temperature of 40°C and a solution to sample ratio of 8. At a temperature of 25°C, the salt content increased from 33.68 to 43.47 with an increase in solution to sample ratio from 4 to 6. Further increase in solution to sample ratio only marginally increased the salt content to 43.91 %. At a constant solution to sample ratio, the salt uptake increased with the increase in temperature from 25 to 55°C. For example, at a solution to sample ratio 4, the salt uptake increased from 33.68 % to 47.47 %, when the temperature was increased to 55°C (Table 1). Similar trend was observed at the other conditions.

TABLE 2. ANOVA (TWO WAY CLASSIFICATION) OF SALT GAIN AT VARIOUS EXPERIMENTAL CONDITIONS

Source of variation	D.F.	S.S.	M.S.	Fcal	Fcri	Inference
Temperature	2	232.42	116.21	7.52	6.94	Significant
Ratio	2	280.60	140.30	9.08	6.94	Significant
Error	4	61.81	15.45			
Total	8	574.83				

TABLE 3. TEST OF SIGNIFICANCE OF TEMPERATURE ON SALT GAIN

Source temperature, °C	Absolute difference	Critical difference	Inference
25 - 40	5.54	7.715	Insignificant
25 - 25	12.42	7.715	Significant
40 - 55	6.88	7.715	Insignificant

TABLE 4. TEST OF SIGNIFICANCE OF RATIO ON SALT

Source temperature, °C	Absolute difference	Critical difference	Inference
4 - 6	13.68	7.715	Insignificant
4 - 8	6.95	7.715	Significant
6 - 8	6.73	7.715	Insignificant

Analysis of variance (Table 2) indicated that both temperature and solution to sample ratio significantly influenced the salt uptake of osmosed mushrooms. However, an analysis of critical difference (Tables 3 and 4) indicated that difference was significant only between temperatures of 25 and 55°C and solution to sample ratio between 4 and 6.

The increase in salt content with time presented a similar pattern at all temperatures (Fig. 2). During the first 5 min of osmosis, the salt content increased from an initial of 0 % to

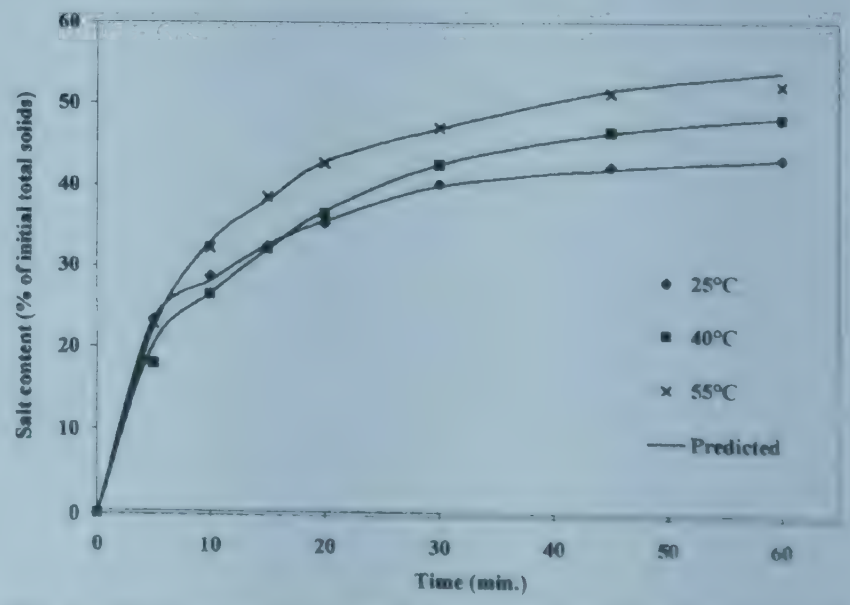

Fig. 2. Performance of Power law model for estimation of salt content at solution to sample ratio 6

values ranging from 15.38 % - 29.64 % depending on the conditions of osmosis. Indicating a faster initial reaction at all temperatures and solution to sample ratios. However, as the time of osmosis progressed, the rate of increase in salt content started to decrease, e.g., at a temperature of 25°C and solution to sample ratio 4, an initial increase of 15 % in the first 5 min. decreased to 4 % (i.e. from 15 to 19 %) in the next 5 min. and continued to increase at a decreasing rate, finally becoming almost 0.5% in the last 5 min. Similar trend was observed for other conditions of osmosis. The rate of salt gain, as proposed by Yang and Le Maguer (1992) was computed as the ratio of difference of salt content and the time interval for each set of consecutive observations. This can be represented by the following equation :

$$\frac{\partial Sc}{\partial t} = \frac{Sc_i}{t} \qquad \text{..... (6)}$$

where,

$\partial Sc/\partial t$ = rate of change of salt content, %/min.

Sc_1 = salt change at any time t, min.

t = time, min.

This ratio represents the rate of salt change with time as a fraction of the total initial weight of the mushrooms. This change of rate systematically decreased with time of osmosis at all conditions. The pattern of change is in agreement with the observations of Yang and Le Maguer (1992). However, the numerical values for the rate of salt change are different, as they had conducted the experiments, using osmoreactor and the process conditions were also different.

The significant change in the salt content of mushrooms with the time of osmosis indicated that the concentration of the solution should also continuously change because of the exchange processes of moisture and salt between mushroom and solution. To analyze this phenomenon, the change in salt concentration in the solution was calculated through mass balance on salt and water as follows :

$$TS = MS + SS \qquad \text{..... (7)}$$

where,

TS = total salt in the system, g

MS = salt in the mushroom at any time t, g

SS = salt in solution at any time t, g

and,

$$TW = MW + SW \qquad \text{..... (8)}$$

where,

TW = total water in the system, g

MW = water in the mushroom at any time t, g

SW = water in solution at any time t, g

The analysis indicated that solution concentration changed significantly with time of osmosis, the change being of the order of upto 2 to 3 % in one hour time. In all cases, the solution concentration continuously decreased with the time of osmosis, indicating that osmosis was an unsteady state process. The extent of change in concentration of the solution depended upon the extent of salt gain by the mushrooms, since salt gain of mushrooms represented the salt loss from the solution (Fig. 3).

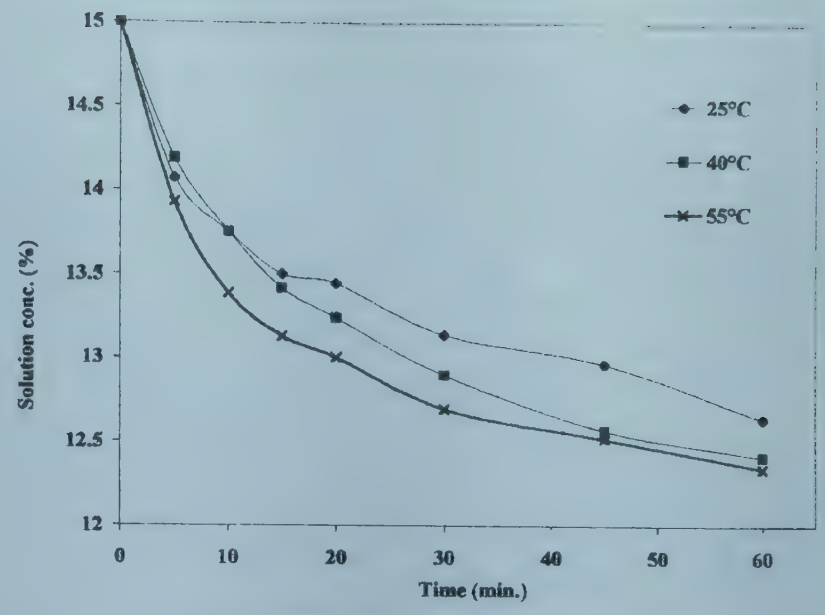

Fig. 3. Solution concentration profiles at solution to sample ratio 6

Salt gain by mushrooms, was as much as 50 % of the final total dry weight. In terms of salt content on the basis of total initial weight of osmosed mushrooms and this worked out to be as high as 6 %. Moreover, after further drying in air or vacuum, only moisture was lost and therefore, the salt content of finally dehydrated mushroom could be as high as 50 %, which was too high for direct consumption. However, it is interesting to note that this excessive salt content readily washes out during the process of washing/rehydration. In fact, the actual salt content of the rehydrated mushroom was well within the acceptable range, as sensory attributes of rehydrated mushrooms were quite comparable to fresh mushrooms with 2 % added salt. This makes osmosis process acceptable because the extra salt helps as a preservative during the storage period and washes out during pre-cooking operations.

The kinetics of salt gain process was studied by fitting power law, log model, generalized exponential model and Page's model stated in equations 1-4 with the incorporation of SC and SR on the LHS in power law, log model and generalized exponential model and Page's model, respectively.

$$SC = \text{Salt change, \%} = \frac{S_t}{W_o} \times 100 \qquad \text{..... (9)}$$

$$SR = \text{Salt ratio,} \frac{S_t - S_f}{S_f} \qquad \text{..... (10)}$$

where,

S_t = salt at any time, t

S_f = final salt gain after 1 h as, g.

W_o = initial weight of solids in the sample, g.

In general, all the four models predicted the data reasonably well, as the value of r^2 ranged from 0.848 to 0.990 and standard error varied from 0.037 to 0.414. However, the power law model best described salt gain kinetics, since the prediction errors were less than 0.25 % (Fig. 2). No systematic

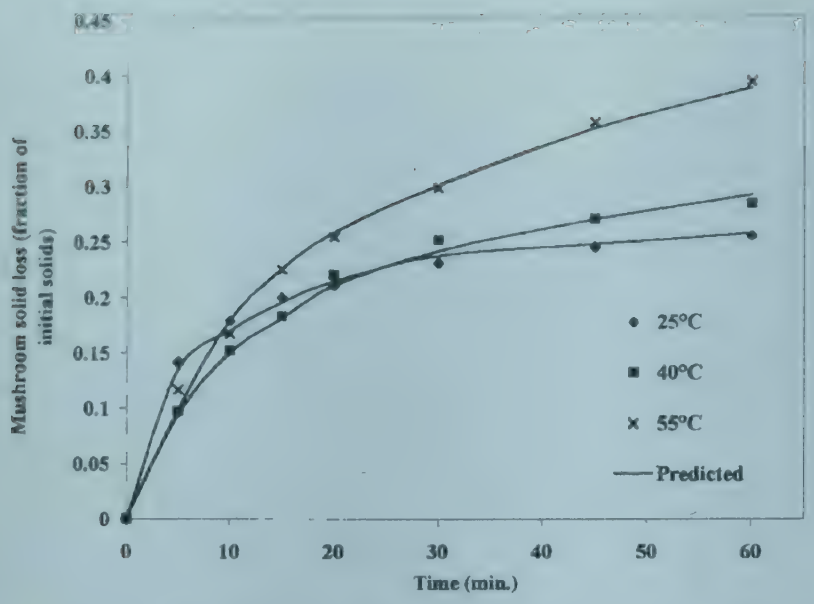

Fig. 4. Performance of Log model for mushroom solids loss at solution to sample ratio 6

pattern could be observed between the model constants and the conditions of osmosis.

An equation of the following form was developed using lumped data for all conditions :

$$SC = at^b T^c R^d \quad (r^2 = 0.950) \qquad \text{..... (11)}$$

where,

SC = salt change, %

t = time, min

T = temperature, °C

R = solution to sample ratio

The constants a, b, c and d were found to be 0.156, 0.373, 0.399 and 0.305, respectively. Temperature had the most significant effect on salt gain, followed by the time of osmosis and solution to sample ratio.

Mushroom solid loss : The fresh mushroom contains about 90% (w.b.) moisture and the remaining 10% as non-salt solids. However, mushroom solid loss due to leaching during osmosis was not considered by the earlier researchers. In order to characterize the mushroom solid loss, the amount of non-salt solids in mushrooms was calculated on the basis of mass balance on mushrooms as follows :

$$MSW = TW - MW - SW \qquad \text{..... (12)}$$

where,

MSW = weight of mushroom solids, g

TW = total weight of mushroom, g

MW = weight of moisture, g

SW = weight of salt, g

The extent of solid loss during osmosis depended upon the temperature as well as the solution to sample ratio. For example, at a temperature of 25°C and solution to sample ratio of 4, the mushroom solid loss was found to be 15.35 % at the end of one hour, whereas at 55°C and solution to sample ratio of 8, the mushroom solid loss was 39.50 % (Table 1), i.e., more than two and half times. Even at a constant solution to sample ratio of 4, the loss of mushroom solids increased from 15.35 % to 30.28 %, when the temperature of osmosis increased from 25 to 55 °C. However, the effect of temperature was less pronounced at solution to sample ratio of 6 and 8, since the loss of mushroom solid increased from 25 to 38 % - 39.18 %, increase being about 50 % at solution to sample ratio of 6, whereas, at solution to sample ratio of 8, the corresponding values were 29.56 % and 39.50 %, representing an increase of about 34 %, being much lower than the increase at solution to sample ratio of 4. Similarly, loss of mushroom solids was maximum at solution to sample ratio of 8 for all temperatures.

The increase in mushroom solids loss with time presented a similar pattern at all temperatures. During the first 5 min. of osmosis, the loss was 3.4 % - 16.36 %, depending upon the conditions of osmosis. This indicated that initial loss of mushroom solids was very fast, indicating a declining rate of loss of mushroom solids (Fig. 4). For example, at a temperature of 25°C and solution to sample ratio of 6, compared to an initial loss of 14 % in the first 5 min. it was only 4 % in the next 5 min. The increase in solid loss continued at a decreasing rate, finally becoming almost 0.3 % in the last 5 min. Similar trend was also observed for other conditions of osmosis.

Kinetics of mushroom solid loss during osmosis was carried out using regression analysis of the following models as given in equations 1-4. The LHS of power law and log models was MS/MS_0 (mushroom solid loss at any time t, g/ mushroom solid present initially, g) and that of generalized exponential and Page's model was MSR (mushroom solid ratio):

All the models described the kinetics of mushroom solids loss reasonably well since r^2 varied from 0.912 to 0.999 and standard error from 0.004 to 0.176. However, log model described the experimental values most accurately. The prediction errors were less than 0.25 %. A generalized model of the following form could incorporate all the variables.

$$MS = a + b\log t + c\log T + d\log R \quad (r^2=0.845) \qquad \text{..... (13)}$$

The model was superior to the generalized power law model. The values of a, b, c and d were - 0.589, 0.184,0.218 and 0.283, respectively.

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RESEARCH PAPER

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A Study of the Digestive Stimulant Action of Select Spices in Experimental Rats

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A few spices, which are commonly employed to improve digestion in traditional systems of medicine were examined for their possible influence on digestive enzymes of pancreas and small intestine in experimental rats. In one set of animals, the spices-ajowan, fennel, coriander, onion, garlic and mint were given through the diet for 8 weeks. In another experiment, the same spices were administered orally as an appropriate single dose to animals. Pancreatic trypsin was significantly stimulated by all the dietary spices examined except mint, while chymotrypsin was stimulated by coriander and onion. These two spices also had a significant stimulating influence on intestinal disaccharidases and alkaline phosphatase. Most of the spices tested in this study showed significant enhancing effect on intestinal enzymes, particularly lipase and amylase, when given as a single oral dose, while similar beneficial effects were not observed on pancreatic enzymes. Among the spices examined, onion produced a pronounced stimulation of a majority of digestive enzymes of pancreas and small intestine. The positive influences on the pancreatic and intestinal digestive enzymes exerted by spices could contribute to their well recognised digestive stimulant action.

Keywords : Spices, Digestive enzymes of pancreas and intestine.

The digestive stimulant action of spices has been recognised since a long time. Several pharmacological preparations available to correct digestive disorders employ certain spices, besides other plant substances. Spices such as mint, garlic, ajowan, fennel, and coriander are the usual ingredients of such digestive stimulant preparations both commercial and as home remedies. Spices have been generally believed to intensify salivary flow and gastric juice secretion, thereby aiding digestion (Glatzel 1968). Earlier studies from this laboratory have revealed that certain spices or their active principles stimulate bile flow and increase biliary bile acids, which have an important role in digestion and absorption of food lipids (Ganesh Bhat et al. 1984, 1985; Sambaiah and Srinivasan 1991). The digestive stimulatory action of spices is also probably through a stimulation of activities of enzymes that participate in digestion. Recently, it has been observed that several of the commonly used spices have a stimulatory influence on digestive enzymes of pancreas and intestinal mucosa in experimental rats (Kalpana Platel and Srinivasan 1996, 2000 a). In the present paper, the influence of dietary intake as well as a single dose administration of few select spices, which are employed to aid digestion in the traditional system of medicine on the digestive enzymes of pancreas and small intestine in albino rats is reported.

The spices studied were: Ajowan (*Trachyspermum ammi*), Fennel (*Foeniculum vulgare*) Coriander (*Coriandrum sativum*), Onion (*Allium cepa*), Garlic (*Allium sativum*), and mint (*Mentha spicata*). Ajowan, being carminative and stomachic, is employed as ingredient of medicinal preparations against digestive disorders, while fennel with its sweet aromatic flavour, commonly chewed after meals is a useful carminative to relieve flatulence (Nadkarni 1976). Coriander seeds are used as stimulant and carminative agents useful in flatulence, indigestion and intestinal disorders; similarly, mint is used as stomachic, tonic, carminative, anti-spasmodic and anti-helmenthic (Nadkarni 1976). Garlic is also considered as carminative, anti-helmenthic, diuretic, and tonic besides being hypolipidemic, while onion, the other *Allium* spice, which is liberally consumed as a vegetable, has hypolipidemic and hypoglycemic potentials (Nadkarni 1976).

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Materials and Methods

Spices ajowan, fennel, and coriander were procured from the local market, cleaned and powdered to pass through a 50 mesh sieve. Garlic and onion obtained locally were freeze, dried; fresh mint leaves were air-dried and the dried spices were powdered. Benzoyl-L-arginine-p-nitroanilide, succinyl-phenylalanine-p-nitroanilide, enterokinase, diethyl dithiocarbamate and 2,4-dinitro-salicylic acid were procured from M/s Sigma Chemical Co., USA. All other chemicals and fine chemicals used were of analytical grade.

Dietary study : Groups of 8 female Wistar albino rats weighing 120 ± 5 g were housed in individual cages and the animals had free access to food and water. The basal diet consisted of (%): casein, 21; cane sugar, 10; corn starch, 54; refined peanut oil, 10; NRC-vitamin mixture, 1 and Bernhard-Tommarelli salt mixture, 4. The various spice diets consisted of respective spices incorporated into this basal diet, substituting an equivalent amount of corn starch at the following levels (%): ajowan (0.2), fennel (0.5), coriander (2.0), onion (2.0), garlic (0.5), and mint (1.0). The rats were thus maintained on *ad libitum* spice diets for 8 weeks.

At the end of the feeding trial, the animals were sacrificed under light ether anaesthesia. Pancreas and small intestine (20-25 cm long segments between jejunum and caecum leaving about 5 cm on either side) were immediately excised. Intestines were flushed with ice-cold 0.9% saline. Intestinal segments were cut open longitudinally and mucosa was scraped with a microscopic slide (Ravinder et al. 1989). The pancreatic tissue samples and intestinal mucosal scrapings were homogenized in ice-cold 0.9% saline and used for various enzyme assays.

Single dose study : Female Wistar albino rats weighing

around 200 g were used in this experiment. The test spices were administered intra-gastrically to overnight-fasted animals as 1 ml suspension in 10 % starch solution at the following doses(mg/kg b.w.):ajowan (100), fennel (250), coriander (500), and fresh onion (2.5 g). A parallel group of control rats received 1 ml 10 % starch solution. Animals were sacrificed under light ether anaesthesia, 3 h after the administration of spices. Pancreas and small intestine were excised immediately and processed as described above.

Standard methods were employed for determining the activities of lipase (Bergmeyer 1974 a), amylase (Bergmeyer 1974 b), trypsin and chymotrypsin (Lewis et al. 1988; Bergmeyer 1984). Lipase was assayed by aerobically incubating the pancreatic homogenate with olive oil suspension in saline in a buffered medium of pH 8.5 and reacting the released free fatty acids with copper nitrate; the copper bound free fatty acids extracted into chloroform were then determined by a colour reaction with diethyl dithiocarbamate (Bergmeyer 1974 a). Activity of amylase was estimated by incubating the pancreatic homogenate with starch solution at pH 6.9 and reacting the released maltose with dinitrosalicylic acid (Bergmeyer 1974 b).

Trypsin and chymotrypsin were determined using benzoyl-L-arginine-p-nitroanilide and succinyl phenylalanine-p-nitroanilide, respectively as substrates (Bergmeyer 1984) after pre-activation with enterokinase according to the procedure of Lewis et al (1988). Disaccharidases - sucrase, lactase and maltase were assayed by incubating the mucosal preparation with the respective disaccharide in maleate buffer (pH 6.0) and measuring the relased glucose by the glucose oxidase method (Bergmeyer 1974 c). Alkaline and acid phosphatases in the mucosal preparations were measured using sodium β-glycerophosphate as the substrate at appropriate pH and measuring the released inorganic phosphorus by the method of Hubscher and West (1965).

Protein content was determined by the modified phenol procedure using bovine serum albumin as standard (Hartree 1972). Statistical analysis of the analytical data was done using Students' t-test and a 'P' value < 0.05 was considered significant (Snedecor and Cochran 1976).

Results and Discussion

In the present study, spices/spice active principles were fed to animals at levels, corresponding to about 5 times the average human dietary intake of these spices. The levels used were based on calculated dietary intake of spices in the form of curry powder and on dietary survey conducted in India (Thimmayamma et al. 1983). Food intake was essentially similar in various spice-fed groups and corresponding control groups. Similarly, the gains in body weights during 8 weeks spice treatment were comparable to controls (data not given).

Influence of dietary spices on pancreatic digestive enzymes is shown in Table 1. It is evident that the dietary intakes of spices - fennel, ajowan, coriander, garlic and onion significantly enhanced the activity of trypsin. The stimulation of this enzyme activity was highest by fennel, being 98 % of the control value, followed by ajowan (48 %), coriander (34 %),

TABLE 1. INFLUENCE OF DIETARY SPICES ON PANCREATIC DIGESTIVE ENZYMES

Diet group	Lipase[1]	Amylase[2]	Trypsin[3]	Chymotrypsin[3]
Control	42.3 ± 2.24	55.0 ± 4.0	2.76 ± 0.11	3.86 ± 0.15
Ajowan	53.1 ± 0.97*	60.0 ± 3.0	4.08 ± 0.39*	4.05 ± 0.13
Fennel	35.7 ± 2.56	62.5 ± 4.0	5.46 ± 0.43*	3.76 ± 0.14
Coriander	43.5 ± 2.47	59.5 ± 1.5	3.69 ± 0.26*	4.30 ± 0.13*
Mint	24.6 ± 2.85**	48.6 ± 2.3	2.52 ± 0.14	3.52 ± 0.12
Garlic	24.1 ± 1.36**	52.1 ± 2.9	3.27 ± 0.11*	3.67 ± 0.32
Onion	39.8 ± 0.16	73.5 ± 1.0*	3.14 ± 0.16*	4.45 ± 0.17*

Values are mean ± SEM of 8 animals per group
[1]:umole FFA/min/mg protein; [2]: nmole maltose/min/mg protein
[3]: nmole p-nitroaniline/min/mg protein
* Significant increase over control
** Significant decrease compared to control

TABLE 2. INFLUENCE OF SINGLE ORAL DOSE OF SPICES ON PANCREATIC DIGESTIVE ENZYMES

Diet group	Lipase[1]	Amylase[2]	Trypsin[3]	Chymotrypsin[3]
Control	41.3 ± 2.19	46.5 ± 1.3	3.15 ± 0.18	3.52 ± 0.36
Ajowan	38.6 ± 2.72	23.3 ± 1.5**	3.73 ± 0.26	3.88 ± 0.13
Fennel	44.2 ± 5.19	58.8 ± 4.8*	3.70 ± 0.29	2.99 ± 0.25
Coriander	48.1 ± 3.97	27.7 ± 2.6**	1.84 ± 0.16**	4.03 ± 0.11*
Onion	38.4 ± 1.62	47.5 ± 3.5	2.21 ± 0.06**	3.67 ± 0.24

Values are mean ± SEM of 8 animals per group
[1]: umole FFA/min/mg protein; [2]: nmole maltose/min/mg protein
[3]: nmole p-nitroaniline/min/mg protein
* Significant increase over control
** Significant decrease compared to control

garlic (30 %) and onion (14 %). Dietary onion and coriander also stimulated chymotrypsin to the extent of 15 and 11 %, respectively. Pancreatic amylase was stimulated by only onion (34 %), while lipase was stimulated only by ajowan (26 %) among the dietary spices examined. On the contrary, the activity of lipase was lowered by dietary mint and garlic to an extent of about 42 %.

The beneficial stimulatory influence of spices on pancreatic enzymes by dietary treatment was, however, not evident, when the same spices were administered orally only once (Table 2). The only stimulatory influence was on the activity of amylase by fennel (26 % increase) and chymotrypsin by coriander (15 % increase). On the other hand, single doses of ajowan and coriander brought about 40-45 % decrease in the activity of pancreatic amylase. A similar decrease in the activity of trypsin was observed in coriander and onion-administered animals.

Tables 3 and 4 depict the influence of dietary spices on the terminal digestive enzymes of small intestinal mucosa. The activities of disaccharidases - sucrase, lactase and maltase were prominently stimulated by dietary coriander and onion. Lactase activity was nearly 3-fold in animals fed coriander, while the corresponding values for sucrase and maltase were 47 % and 22 % higher, respectively. The stimulation of sucrase and lactase by dietary onion was about 90 %, while that of maltase was 50 %. These two spices also stimulated the activity of alkaline phosphatase by 54 and 73 %, respectively. Dietary onion also enhanced the activity of intestinal amylase to an extent of 24 %. Contrary to a beneficial stimulatory effect, dietary mint showed an undesirable lowering influence

TABLE 3. INFLUENCE OF DIETARY SPICES ON DIGESTIVE ENZYMES OF INTESTINAL MUCOSA

Diet group	Lipase[1]	Amylase[2]	Alkaline phosphatase[3]	Acid phosphatase[3]
Control	748.4 ± 36.0	44.0 ± 1.6	22.3 ± 1.14	0.91 ± 0.08
Ajowan	624.4 ± 31.8**	40.0 ± 0.6	22.1 ± 0.84	0.66 ± 0.09**
Fennel	542.4 ± 9.4**	40.0 ± 1.6	20.1 ± 1.84	0.61 ± 0.03**
Coriander	757.2 ± 64.8	49.6 ± 3.2	34.4 ± 3.22*	1.01 ± 0.07
Mint	589.7 ± 34.6**	48.6 ± 1.5	14.6 ± 0.49**	0.76 ± 0.04
Garlic	674.6 ± 96.8	45.5 ± 2.3	24.8 ± 1.90	0.98 ± 0.03
Onion	703.8 ± 99.4	54.4 ± 2.4*	38.6 ± 1.39*	0.89 ± 0.10

Values are mean ± SEM of 8 animals per group
[1] nmole FFA/min/mg protein; [2]: nmole maltose/min/mg protein
[3]: µmole pi/hr/mg protein
* Significant increase over control
** Significant decrease compared to control

TABLE 4. INFLUENCE OF DIETARY SPICES ON DISACCHARIDASES OF INTESTINAL MUCOSA

Diet group	Sucrase	Lactase	Maltase
Control	77.6 ± 2.64	29.5 ± 2.37	513.5 ± 28.4
Ajowan	87.1 ± 2.12*	23.5 ± 1.90**	520.7 ± 10.2
Fennel	96.5 ± 3.44**	24.4 ± 1.66	541.2 ± 6.4
Coriander	114.3 ± 5.52*	86.7 ± 6.03*	625.4 ± 17.9*
Mint	48.7 ± 4.62**	7.31 ± 0.96**	427.6 ± 25.8**
Garlic	87.6 ± 7.74	30.3 ± 5.65	489.4 ± 10.3
Onion	147.9 ± 6.24**	55.1 ± 3.31*	769.2 ± 30.6*

Values are mean ± SEM of 8 animals per group
Activity Units : nmole glucose/min/mg protein
* Significant increase over control
** Significant decrease compared to control

TABLE 5. INFLUENCE OF SINGLE ORAL DOSE OF SPICES ON DIGESTIVE ENZYMES OF INTESTINAL MUCOSA

Diet group	Lipase[1]	Amylase[2]	Alkaline phosphatase[3]	Acid phosphatase[3]
Control	735.3 ± 054.0	46.2 ± 0.5	23.4 ± 0.78	1.03 ± 0.04
Ajowan	1566.6 ± 241.5*	80.3 ± 5.6*	47.8 ± 5.52*	1.22 ± 0.12
Fennel	1248.1 ± 128.6*	81.3 ± 3.6*	28.9 ± 3.22	1.20 ± 0.04*
Coriander	771.0 ± 117.3	63.4 ± 3.9*	21.4 ± 1.94	0.82 ± 0.04**
Onion	1791.8 ± 164.2*	103.7 ± 6.4*	30.3 ± 1.48*	1.21 ± 0.02*

Values are mean ± SEM of 8 animals per group
[1]: nmole FFA/min/mg protein; [2]: nmole maltose/min/mg protein
[3]: µmole pi/hr/mg protein
* Significant increase over control
** Significant decrease compared to control

TABLE 6. INFLUENCE OF SINGLE ORAL DOSE OF DIETARY SPICES ON DISACCHARIDASES OF INTESTINAL MUCOSA

Diet group	Sucrase	Lactase	Maltase
Control	63.1 ± 3.65	28.4 ± 1.47	522.4 ± 32.8
Ajowan	76.7 ± 0.73*	28.3 ± 0.63	514.2 ± 34.7
Fennel	68.2 ± 4.33	30.4 ± 3.09	554.4 ± 16.9
Coriander	38.8 ± 1.51**	15.9 ± 1.04**	435.9 ± 10.6**
Onion	86.1 ± 4.14*	35.5 ± 2.28*	653.4 ± 18.3*

Values are mean ± SEM of 8 animals per group
Activity Units : nmole glucose/min/mg protein
* Significant increase over control
** Significant decrease compared to control

on most of the enzymes of the intestinal mucosa. This effect was most pronounced in the case of lactase, where the enzyme activity was decreased by as much as 75 %. Dietary ajowan and fennel also showed a similar decreasing effect on intestinal lipase and acid phosphatase, while these spices enhanced the activity of only sucrase (12 and 24 %).

Activities of digestive enzymes of intestinal mucosa in animals, administered spices as a single oral dose are given in Tables 5 and 6. Onion exerted a beneficial stimulatory effect on the activities of all the intestinal enzymes, when given as a single oral dose. The influence on lipase and amylase was enormous with over 100 % stimulation, while those on the other enzymes were in the range of 18-35 %. Contrary to its beneficial effect, when consumed through diet, coriander had a decreasing influence on most of the digestive enzymes of small intestinal mucosa, when its intake was restricted to a single oral dose. The activity of amylase was enhanced by all the four spices tested, when administered orally, the extent of enhancement being 124, 76, 74 and 37 % by onion, fennel, ajowan and coriander respectively. Single dose each of ajowan and fennel also resulted in a significant stimulation of intestinal lipase and phosphatase. The increases in lipase activities were 113 and 70%, respectively. The increase in the activity of alkaline phosphatase by ajowan was over 100 %. In addition, ajowan also stimulated sucrase activity to a small extent (22%).

Any food additive or adjunct, which has a digestive stimulant action may exert such an effect through a positive influence on the digestive secretions (gastric, bile, pancreatic) or specifically the enzymic content of gastric, pancreatic and intestinal juices. In addition, they may also increase biliary secretion of bile acids, which are known to play a major role in fat digestion and absorption. The overall digestive stimulatory influence could be a combination of these two mechanisms or might involve any one of these independent mechanisms.

Since the contributions of digestive enzymes of pancreas and the terminal enzymes of small intestinal mucosa to the overall process of digestion are predominant, this study was confined to the examination of the influence of spices on these enzymes. Data on the influence of single dose intake of mint and garlic on digestive enzymes of pancreas and small intestine have been reported by Sharathchandra et al (1995).

Among the 6 spices tested, only dietary ajowan had an enhancing influence on the activity of pancreatic lipase, which plays a vital role in digestion of fat. Intestinal lipase, which was incidentally stimulated by onion, ajowan and fennel, when given as a single dose was distinct from pancreatic lipase, and had been claimed to significantly supplement pancreatic lipase in the hydrolysis of dietary fat, specifically mono-glycerides (Di Nella et al. 1960). Intestinal lipase probably assumes a greater role in the digestion of fat, when the titres of pancreatic lipase are limiting. Dietary mint produced a significant decrease in pancreatic lipase activity, which was contrary to its effect when given as a single oral dose (Sharathchandra et al. 1995). Dietary garlic also produced a similar decreasing effect on pancreatic lipase. The stimulatory influence of mint and garlic given as a single oral dose on the activity of intestinal lipase

(sharathchandra et al. 1995) however, was not evident, when these two spices were given through the diet.

While both pancreatic and intestinal amylases were stimulated by dietary onion, all the tested spices enhanced the activity of intestinal amylase alone, when ingested as a single oral dose. Stimulation by spices of pancreatic proteases-trypsin and chymotrypsin, which play a major role in digestion of food proteins was confined to dietary treatment alone, such an effect being more on trypsin than on chymotrypsin.

Among the terminal digestive enzymes of intestinal mucosa, disaccharidases aid in the completion of carbohydrate digestion. The intake of onion either dietary or as a casual single dose brought about a beneficial stimulation of all the disaccharidases - sucrase, lactase, and maltase, the effect being more pronounced with a continued intake of the spice. Coriander had a similar enhancing effect on all the disaccharidases, only when consumed through the diet. Dietary mint had a decreasing effect on the activities of disaccharidases, similar to its effect on the activity of lactase, when administered as a single oral dose (Sharathchandra et al. 1995).

The spices studied in this investigation are extensively used to correct digestive disorders in Indian households. Stimulation of digestive enzymes is an important factor that contributes to the digestive stimulant action of these food additives. The present study documents the favourable influence of onion in particular on a majority of digestive enzymes of both the pancreas and small intestine. This beneficial influence of onion on pancreatic digestive enzymes was more evident by a continued intake of this spice, while intestinal enzymes were stimulated even with one-time exposure. Pancreatic trypsin, an important protease, was generally stimulated by these spices during their continuous intake and a majority of them also stimulated intestinal disaccharidases.

Yet another observation was that, while the pancreatic enzymes were not influenced by the test spices given as a single oral dose, this one-time exposure had a significantly enhancing effect on intestinal enzymes, particularly lipase and amylase. The transient presence of the spice at the site of action may have been responsible for such a differential effect observed in this study.

Conclusions

Findings of the present study have clearly indicated the favourable influence of a few spices employed in traditional remedies for improved digestion, on the activity of trypsin of pancreatic tissue and disaccharidases of small intestinal mucosa. In addition to this, beneficial influence on bile secretion with a higher bile acid content could be a mechanism through which spices exert their digestive stimulant action. Investigations to this effect in this laboratory have shown that dietary curcumin, capsaicin, ginger, fenugreek, ajowan, coriander, mint and onion significantly enhance the secretion of bile acids (Ganesh Bhat et al. 1984, 1985; Sambaiah and Srinivasan 1991; Kalpana Platel and Srinivasan 2000 b). Thus, the present study has evidenced that onion, among the spices tested, produces a pronounced stimulation of a majority of digestive enzymes of pancreas and small intestine, which could be complementary to the stimulatory influence on onion on bile acids observed earlier (Sambaiah and Srinivasan 1991), contributing to the overall digestive stimulant action of this spice. It is interesting to note that garlic, which also belongs to the *Allium* species, did not seem to beneficially influence either digestive enzymes, as observed in the present study or bile acid secretion (Kalpana Platel and Srinivasan 2000 b).

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RESEARCH NOTE

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Tray Over-wrapping of 'Mosambi' Sweet Orange

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'Mosambi' sweet orange (*Citrus sinensis* Osbeck) fruits were treated with three pre-harvest sprays of carbendazim (500 ppm) at 15 days interval before harvest, degreened with ethylene gas, washed (chlorinated water 1000 ppm) and treated with carbendazim (2000 ppm) on brush rollers before tray over-wrapping. 'Cryovac' heat-shrinkable films (polyolefin material) and LDPE heat-shrinkable and stretchable (Linear low density polyethylene) cling films were wrapped over trays, holding 6 fruits and stored at 25 ± 5°C and 45 ± 5 % relative humidity in vented corrugated fibre-board master boxes (6 trays in a master box) for analysis after 20 and 40 days. Non-wrapped fruits served as control. Cryovac film-wrapped (BDF 2001, 30 µm thick; D-955, 15 and 25 µm thick) and stretchable film (15 µm) wrapped fruit had significantly lower (1/9 – 1/16th) weight loss compared with that of non-wrapped fruits after 20 and 40 days. In LDPE (50µm) heat-shrinkable film wrapped fruit, weight loss was 1/16 – 1/20th as against control. There was no spoilage in any treatment up to 40 days except D-955 (15 µm) film (6.01 %) after 20 days. Rind colour development was not affected by wrapping of fruit in trays. Fruit firmness decreased slightly in most of the treatments after 20 and 40 days except in non-wrapped and SM-250 (perforated) film wrapped fruit, whose higher firmness was due to fruit dessication. Juice content significantly dropped in non-wrapped and SM-250 film wrapped fruits due to water loss. Ascorbic acid content declined with non-significant differences among the treatments; loss being more in control and SM-250 film wrapped fruit. Appearance and flavour were not acceptable after 20 days in control and SM-250 film wrapped fruit. Appearance was attractive (score more than 7 on 1-9 scale) and flavour was acceptable (score more than 7 on 1-9 scale) after 40 days in fruit wrapped with 'Cryovac' films especially D-955, LDPE heat-shrinkable and stretchable cling film.

Keywords : *Citrus sinensis* Osbeck, 'Mosambi', Tray over-wrap, Heat-shrinkable and stretchable films, Shelf life.

In India, sweet oranges (*Citrus sinensis* Osbeck) are marketed under hot and dry ambient conditions, leading to fruit desiccation, loss of juice, loss of weight and rapid deteriotation in overall quality. These fruits have fairly high content of ascorbic acid and mostly utilized for consumption as fresh fruit. 'Mosambi' sweet oranges occupy more than 30,000 ha area in Maharashtra alone with annual production of nearly 20,0000 metric tonnes. The sweet oranges are also produced in Andhra Pradesh, Madhya Pradesh, Rajasthan, Punjab and West Bengal with total production of more than 90,0000 tonnes.

Film wrapping of mandarins (*Citrus reticulata* Blanco), has been very promising with respect to controlling water loss and spread of decay, retention of fruit shape without adverse effect on flavour and colour development (Ladaniya et al. 1997). In fact, this technique provides an alternative to the wax coating. The film also provided protection from dust and thus resulted in hygienic, attractive and very handy consumer pack for retail outlets most suitable for tropical Indian marketing conditions.

The storage of 'Mosambi' fruits in vented polyethylene bags has been reported to reduce water loss considerably, while fungicides were effective in minimizing rotting (Kumbhare and Chaudhari 1979). The reports on tray over - wrapping with heat-shrinkable films and stretchable cling films for 'Mosambi' sweet oranges are not available. Hence, the present work was undertaken to study the shelf life of degreened 'Mosambi' sweet orange tray-over wrapped with heat-shrinkable and streatchable films at ambient conditions. Most films used in this study have high CO_2 and O_2 premeability, low water vapour transmission rate and are suitable for use in automatic machines (Table 1). The stretchable films can be wrapped manually, thus minimizing capital investments on machinery.

During *Ambia* (Spring blossom) crop harvesting season, spray treated (3 pre-harvest sprays of carbendazim at 500 ppm at an interval of 15 days) 'Mosambi' fruits were harvested at maturity. Fruits were subjected to degreening (5-10 µl/lit C_2H_4 at 28-30°C, 90-95% RH) for 48 h in the degreening chamber (Ladaniya 1999) for yellow colour development before washing with chlorinated (1000 ppm chlorine) water and spray treatment with carbendazim (at 2000 ppm) on horse-hair roller conveyer. Surface-dried fruits were tray over-wrapped as follows :

Fruits were placed in a tray of corrugated fibre board and various heat-shrinkable films were loosely packed around the trays with a sealer (Weldotron Model 6300 L) before wrapping in a heat-shrink tunnel (Weldotron Model 7121 A). The LDPE

TABLE 1. PROPERTIES OF THE FILMS USED FOR TRAY WRAPPING OF 'MOSAMBI'

Films	Thickness, µm	WVTR,* g/m²/day	Permeability, cc/m²/day		Density, g/cc
			O_2	CO_2	
Heat-shrinkable films					
Polyethylene (LDPE)	50	9.90	4125	16175	0.91
BDF-2001 (multilayered co-extruded polyolefin)	30	12.66	3	12	0.96
D-955 (cross-linked polyolefin)	25	13.66	4941	19764	0.92
D-955 (cross-linked polyolefin)	15	23.00	8548	34192	0.92
SM-250 (polyolefin) (0.1 mm² perforations; 9 perforations/cm²)	35	250.00	–	–	0.91
Stretchable cling film					
Linear - low density polyethylene (LLDPE)	15	23.90	9875	39025	0.92

Note : Based on information supplied by the manufacturers before shrinkage of the film and at 21-23°C and 60-65% relative humidity
*WVTR - Water vapour transmission rate

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heat-shrinkable film was wrapped at 147°C in 15 sec. The BDF-2001 and D-955 films were wrapped at 106°C in 30 sec, while SM-250 film (micro perforations of 0.01 mm² size) was wrapped at 147°C in 20 sec. The stretchable film was manually wrapped around the trays. Unwrapped fruits served as control. The trays of over-wrapped fruits were placed in the vented (5% side area punched for vents) CFB master-boxes (6 trays in each box) and stored at 25 ± 5°C and 45 ± 5% relative humidity for analysis after 20 and 40 days.

Fruit firmness was measured (after removing film wrapper) with the help of hand - held pressure tester (Effigy model 011) using round plunger (8 mm diameter) and force expressed as Newtons (N). Juice was extracted with power - operated reamer and total soluble solids (TSS) were measured with a hand refractometer, whereas titratable acidity (expressed as citric acid) and ascorbic acid contents in the juice were measured using titrimetric methods as described by Ranganna (1986). Before wrapping, rind colour of three fruits was recorded with colour gard systems 1000/05 (Pacific scientific, USA) for tristimulus of L*, a* and b*. Fruits under various treatments were again measured for colour at each interval. The measurements were according to CIE (Commission Internationals and Eclairage) system as L* (Lightness or darkness; 0 = black, 100 = white), a* (bluish-green/red purple hue component) and b* (yellow/blue hue component). The a* and b* co-ordinates were transferred into hue angle (arc tangent b/a) and saturation index i.e. chroma $\sqrt{a^2 + b^2}$ according to Mc Guire (1992). The appearance and flavour of wrapped and unwrapped fruits were evaluated in a preference test at individual station by a trained 15 member preference panel (Heintz and Kader 1983). From each treatment, a set of 2 whole fruits and 2 quarters of cut fruits were given to each individual panelist drawn from the staff of the Centre. A 9 - point Hedonic scale was given on score card (1 - dislike extremely; 5 - neither like nor dislike; 9 - like extremely) to the panelist. The numerical data were subjected to analysis of variance. The percent weight loss and extent of decay were recorded during storage and decay causing organisms were identified.

There were seven treatments, twelve replications in each treatment with 6 fruits in each replicate. Treatments were organized in a completely randomized design and data were subjected to analysis of variance (P = 0.05).

TABLE 2. PERCENT WEIGHT LOSS AND DECAY IN TRAY OVER-WRAPPED 'MOSAMBI' SWEET ORANGE FRUITS

Treatment	Weight loss, %		Decay, %	
	Days in storage			
	20	40	20	40
Heat-shrinkable film				
LDPE (50 µm)	0.64	1.36	0.00	0.00
BDF 2001 (30 µm)	0.72	1.47	0.00	0.00
D-955 (25 µm)	0.87	2.00	0.00	0.00
D-955 (15 µm)	1.48	2.76	6.01	0.00
SM-250 (35 µm)	11.96	22.30	0.00	0.00
Stretchable film				
LLDPE (15 µm)	2.23	3.65	0.00	0.00
Control (non-wrapped)	10.56	26.66	0.00	0.00
SE ±	0.43	0.83	2.27	–
CD (P = 0.05)	1.38	2.57	NS	–

Physiological loss in weight and decay : In tray over-wrapped fruits, relatively thicker LDPE (50 µm) heat-shrinkable film controlled weight loss more effectively as compared with thinner films i.e., D-955 (15 µm) and strecthable (15 µm) cling film (Table 2). The weight loss was relatively higher in stretchable cling film wrapped trays and this can be attributed to comparatively higher water vapour transmission rate (23.90 g/m²/day) among all the non-perforated films (Table 1). As the storage period extended up to 40 days, weight loss increased with the difference in wrapped and non-wrapped fruits further widening and weight loss being as high as 20 times in control as compared with that of LDPE (50 µm) heat-shrinkable film wrapped fruits. The perforated SM-250 film was completely ineffective in retaining freshness due to very high (250 g /m²/day) water vapour transmission rate in this film.

After 20 days, decay was not recorded in any of the treatment except D-955 (15 µm). There was no decay after 40 days. The decay was due to sour rot caused by *Geotrichum candidum*. The pre-and post-harvest treatments with carbendazim effectively controlled stem-end rots. The response of fungicide treatment in this study was quite encouraging, since the ethylene exposure to citrus fruits has been reported to incite stem-end rots (Eckert and Brown 1986). Pre-and post-harvest fungicide treatments with carbendazim have consistently minimized decay in mandarins (Ladaniya et al. 1997; Sonkar and Ladaniya 1998).

Rind colour : Colour development further continued in tray over-wrapped and non-wrapped fruits during storage (Table 3). The lightness (L*) increased with increase in storage period in all the treatments except non-wrapped (control) and SM-250 film wrapped fruits, which lacked brightness of colour with decrease in L* value due to shrivelling. The colour intensity also increased in most of the treatments with consequent reduction in hue (h^0) angle except in unwrapped fruits and SM-250 film where colour intensity i.e. C* (Chroma) decreased. The 'Cryovac' and LDPE (heat shrinkable and stretchable) wrapped fruits were vivid yellow in colour, while non-wrapped (control) fruits had reddish orange tinge due to water stress, indicating significantly lower hue angle after 40 days. After degreening, there was a carry over effect of ethylene, which resulted in continued colour development and it was not adversely affected by film wrapping. In ethylene-treated fruits, wrapping with 'Cryovac' films did not affect colour development at 20°C (Eaks 1991). The present findings indicated that O_2 transmission rate of the films used was sufficient to maintain colour change. Depleted O_2 rather than high CO_2 has been found to inhibit colour development in citrus (Petracek and Montalvo 1997).

Fruit firmness decreased in all the treatments after 20 days with non-significant difference (Table 4). Non-wrapped (control) and SM-250 film-wrapped fruits continued to lose water rapidly up to 40 days, resulting in drying/toughening of peel and significant increase in firmness. Non-perforated 'Cryovac' and LDPE films provided moisture barrier and thus resulted in retention of 95-96% of the initial juice content as compared with 84-85% in non-wrapped and SM-250 film wrapped fruits after

TABLE 3 RIND COLOUR IN TRAY OVER-WRAPPED 'MOSAMBI' FRUITS

Treatment	L*			C*			h°		
	Storage period, days								
	0	20	40	0	20	40	0	20	40
Heat-shrinkable film									
LDPE (50 µm)	80.89	81.62	84.31	65.08	66.90	68.27	88.07	88.03	87.18
BDF 2001 (30 µm)	80.97	81.86	84.51	65.51	67.01	67.77	88.08	87.32	86.49
D - 955 (25 µm)	82.36	84.82	86.16	65.43	67.24	68.24	88.21	87.00	86.56
D - 955 (15 µm)	81.30	82.56	84.84	66.43	67.42	68.36	88.09	87.36	86.56
SM - 250 (35 µm)	80.90	77.10	68.10	66.47	69.85	63.74	88.28	86.91	85.39
Stretchable film									
LLDPE (15 µm)	82.02	83.49	84.36	65.40	65.85	66.85	88.14	86.03	84.68
Control	80.76	77.41	66.86	65.83	69.46	62.79	88.25	84.91	83.10
SE ±	0.45	0.62	0.82	0.01	0.83	1.08	0.36	0.46	0.50
CD at 5%	NS	1.95	2.52	NS	2.56	3.34	NS	1.44	1.57

L* = lightness or darkness (0 = Black, 100 = White); C* = Chroma (0 = lowest intensity, 100 = greatest intensity); h° = hue angle (o° = red purple, 90° = yellow, 180° = bluish-green, 270° = blue)

TABLE 4. PHYSICO-CHEMICAL AND SENSORY ATTRIBUTES IN TRAY OVER-WRAPPED 'MOSAMBI' SWEET ORANGE FRUITS DURING STORAGE

Treatment	Firmness, N		Juice, %		TSS, %		Titratable acidity, %		Ascorbic acid, mg/100 ml		Appearance, score 1-9		Flavour, score 1-9	
	Storage period, days													
	20	40	20	40	20	40	20	40	20	40	20	40	20	40
Heat - shrinkable	(95.89)		(41.18)		(9.95)		(0.30)		(42.79)					
LDPE (50 µm)	88.94	89.59	39.66	39.24	9.06	9.86	0.28	0.27	39.09	37.69	8.16	7.91	7.50	7.25
BDF 2001 (30 µm)	95.81	89.59	38.63	38.16	9.86	9.93	0.30	0.25	42.56	37.91	8.41	8.14	8.26	7.02
D-955 (25 µm)	95.81	90.90	38.35	38.29	9.73	9.33	0.30	0.23	41.46	38.77	8.35	8.14	7.56	7.21
D-955 (15 µm)	97.96	91.55	39.74	39.53	10.00	9.53	0.27	0.26	42.65	40.46	8.35	8.03	8.58	7.50
SM-250 (35 µm)	91.23	103.34	36.58	34.74	10.73	12.53	0.32	0.33	40.26	34.28	5.57	4.98	5.25	3.34
Stretchable film														
LLDPE (15 µm)	93.52	92.21	38.75	38.42	9.53	9.14	0.28	0.26	38.17	37.48	7.57	7.09	7.68	7.22
Control (non-wrapped)	94.50	98.69	37.38	35.12	9.93	10.60	0.38	0.31	40.73	35.49	5.56	4.39	5.50	4.20
SE±	2.77	1.95	1.15	0.98	0.23	0.30	0.28	0.01	1.52	0.96	0.24	0.22	0.12	0.66
CD at P = 0.05	NS	6.03	NS	3.04	NS	0.93	NS	0.05	NS	NS	0.75	0.70	0.39	0.20

Note : Figures in parenthesis indicate initial values (at 0 days)

40 days. Changes in TSS and acidity contents of fruits under various treatments were inconsistent. However, in non-wrapped and SM-250 film-wrapped fruits, TSS or titrable acidity rose significantly after 40 days and this was attributed to excessive loss of water and rise in the level of these soluble constituents of the juice. Ascorbic acid retention was better in non-perforated film-wrapped fruits. Appearance and flavour scores were more than 7 in 'Cryovac' (non-perforated) and LDPE film-wrapped fruits. Due to shrinkage and off-flavour, non-wrapped (control) and SM-250 film wrapped fruits were not acceptable after 20 days. The excessive drying of peel due to transpiration has been reported to increase resistance to gas exchange through peel, which changed the internal atmosphere in Shamouti oranges (Ben Yehoshua 1969). Film-wrapped fruits in the trays were turgid, fresh, shining and acceptable to the panelists during organoleptic evaluation. 'Cryovac' (non-perforated) and LDPE films had sufficiently high CO_2 permeability (Table 1) except BDF 2001. Considering lower respiration rate of 'Mosambi' orange (30-35 mg CO_2/kg/h) at 25-30°C), possibility of CO_2 accumulation inside tray-wrap with consequent off flavour development is less likely. Large scale trial with BDF 2001 to confirm this aspect would be justifiable. Packaging of eight fruits of 'Fremont' mandarin in a cardboard tray over-wrapped by 19 µm thick polyethylene film enabled acceptable fruit quality to be maintained during storage for a week at 20°C after 6 weeks at 6°C (Piga et al. 1996).

The present study has clearly indicated that heat-shrinkable films such as 'Cryovac' (D-955; 25 and 15 µm) and LDPE (50 µm) as well as streatchable cling film (15 µm) can double the shelf life of 'Mosambi' at 25 ± 5°C and 45 ± 5% relative humidity. Unwrapped and perforated film (SM250) wrapped fruits lose freshness and become unacceptable within 20 days, while tray-wrapped fruits are acceptable up to 40 days. Decay can be controlled effectively with pre - and post-harvest fungicidal treatments. Tray over - wrap can be a very handy and hygienic unit from retail marketing point of view.

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RESEARCH NOTE

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Effect of Microwave Heating on Hydroxymethylfurfural Formation and Browning in *Helianthus annuus* and *Eucalyptus lanceolatus* Honey

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The effect of microwave heating on Hydroxymethylfurfural (HMF) formation and colour development, measured as absorbance in *Helianthus annuus* and *Eucalyptus lanceolatus* was studied using regression analysis. Formation of HMF and absorbance increased with the increase in microwave power levels and duration of heating. However, microwave power levels showed a greater effect Both the honey types differed significantly with respect to HMF formation and absorbance under similar microwave heating conditions. *Helianthus annuus* showed higher HMF formation and absorbance value as compared to *Eucalyptus lanceolatus* under similar microwave heating conditions. The regression models for HMF formation and absorbance were significant at $p<0.00$ level and showed R^2 values in the range of 91.5-94.4%. The models developed can be used as a tool to predict changes in colour and HMF content of honey during microwave heating.

Keywords : Honey, Hydroxymethylfurfural, Microwave heating, Colour development.

Honey immediately after extraction is at its best in flavour and colour. But it contains pollens, bits of wax, variable amounts of sugar crystals of dextrose etc. Hence, heat processing of honey is essential to extend its shelf-life, prevent granulation and fermentation (Singh et al. 1988). Uncontrolled heat processing of honey results into hydroxymethylfurfural (HMF) formation and also a darkened colour of honey. Honey darkens during heating due to reaction between reducing sugars and amino groups of the proteins (Maillard reaction). High levels of HMF formation suggest the possibility that the natural honey has been adulterated by invert sugar, prepared by acid hydrolysis of sucrose (Doner 1977). Temperature and duration of heating of honey must be controlled because excessive amount of HMF formation indicates excessive heating and loss of freshness of honey (Isbell et al. 1969; White 1978). Temperature and duration of heating during processing must be controlled considering the floral source from which honey has been extracted (Singh and Bath 1997, 1998). Heating conditions, storage duration and source of honey should be considered together during processing of honey (Gupta et al. 1992; Bath and Singh 1999). Earlier, the effect of convective heating on HMF formation in honey has been studied (Singh and Bath 1997, 1998). The use of microwave heating at small-scale industry for preventing deterioration of honey quality has also been suggested by Ghazali et al (1989). They did not observe any significant changes in pH, total acidity, ash, glucose, fructose and sucrose contents of honey during microwave heating. These authors, however, had not studied the effect of microwave heating on HMF formation in honey.

The present investigation was undertaken to i) study the effect of microwave power levels and heating duration on HMF formation and colour development in *Helianthus annuus* and *Eucalyptus lanceolatus* honey and ii) develop second order polynomials to relate microwave power levels and heating duration with HMF content and colour development in *Helianthus annuus* and *Eucalyptus lanceolatus* honey.

Collection and preparation of samples : Samples of honey were collected to represent *Helianthus annuus* and *Eucalyptus lanceolatus* sources from 1997 season. The sample used in the present investigation was ripe honey, harvested from hives, which were located in the areas, where *Helianthus annuus* and *Eucalyptus lanceolatus* constituted the majore flora for the collection of nectar. Extraction was carried out by filtration and squeezing the honey through muslin cloth and stored in air-tight plastic jars for further analysis.

Microwave heating : Honey sample (5 g) was poured in Teflon beakers and heated at 70, 140, 210 and 280 W for 1, 2, 3 and 4 min in a domestic microwave oven (Kelvinator, Model-T 23). After each test, the door of the microwave oven was kept open for 5 min to facilitate cooling. The power output of the magnetron of microwave oven specified by the manufacture was 700 W and the operating frequency was 2450 MHz.

Analysis : °Brix was determined using an Abbemat Digital Automatic Refractometer (Dr. Wolfgang Kernchen GMBH, Seelze, Germany). Refractive indices of honey types were measured using an Abbemat Refractometer at 20°C and corresponding moisture content (%) was calculated using relationships between refractive index and water content (AOAC 1990). The determination of HMF content of honey samples was carried out using AOAC (1990) methods. To measure colour development, honey samples (1 g) were poured in Teflon beakers and heated at 70, 140, 210 and 280 W for 1, 2, 3 and 4 min and cooled to room temperature. The volume was made up to 10 ml with distilled water and absorbance (O.D.) of each sample was taken at 420 nm using Spectronic-20D (Milton Roy, USA).

Statistical analysis : The second order polynomials were computed using two variables at five levels design by regression to fit a response surface analysis. Microwave power levels and heating duration were used as dependent variables. The significance of each co-efficient in the regression model was determined using t-test. The data reported are average of triplicate observations.

The second order polynomials fitted to the data of *Helianthus annuus* and *Eucalyptus lanceolatus* honey were

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TABLE 1. REGRESSION MODELS FOR ABSORBANCE AS A FUNCTION OF MICROWAVE POWER LEVELS AND HEATING DURATION IN HONEY TYPES

Honey type	Term	Co-efficient	t-value
Helianthus annuus	Constant	0.9783	
	Power level	–0.0041	–3.994***
	Heating duration	–0.1348	–1.887*
	Power level x Power level	0.000	3.683***
	Heating duration x Heating duration	0.0117	0.894*
	Power level x Heating duration	0.0007	4.958***
	R^2	91.9%	
	Model p value	0.000	
Eucalyptus lanceolatus	Constant	1.2589	
	Power level	–0.0070	3.936***
	Heating duration	–0.1959	–2.594**
	Power level x Power level	0.0000	–1.041*
	Heating duration x Heating duration	0.0084	3.268**
	Power level x Heating duration	0.0016	NS
	R^2	94.4%	3.955***
	Model p value	0.000	
	***p<0.005, **p<0.05, *p<0.5		

TABLE 2. REGRESSION MODELS FOR HMF FORMATION AS A FUNCTION OF MICROWAVE POWER LEVELS AND HEATING DURATION IN HONEY TYPES

Honey type	Term	Coefficient	t-value
Helianthus annuus	Constant	0.3158	
	Power level	0.1363	3.957***
	Heating duration	0.9400	NS
	Power level x Power level	–0.0003	–2.86**
	Heating duration x Heating duration	–0.1727	NS
	Power level x Heating duration	0.0075	1.486*
	R^2	93.9%	
	Model p value	0.000	
Eucalyptus lanceolatus	Constant	9.786	
	Power level	–0.051	–3.125**
	Heating duration	–1.324	–1.16*
	Power level x Power level	0.000	3.113**
	Heating duration x Heating duration	0.095	NS
	Power level x Heating duration	0.010	4.14***
	R^2	91.5%	
	Model p value	0.000	
	***p<0.005, **p<0.05, *p<0.5		

computed and equations tabulated are presented in Tables 1 and 2. All the models correlated well with the measured data and were statistically significant. R^2 values for different models ranged between 91.5 - 94.4 %, which represented the proportion of variability of data accounted for by the model (Gardiner 1997). Microwave power level showed the most pronounced effect both in linear and squared terms on HMF formation and absorbance in both the honey types. Duration of heating also showed significant effect on HMF formation and absorbance in *Helianthus annuus* and *Eucalyptus lanceolatus* honey samples. However, the effect was significant in squared terms and at higher p value. Microwave power and duration of heating interacted significantly in effecting the HMF content and absorbance in both the honey types and the effect was more pronounced in *Eucalyptus lanceolatus* honey.

Eucalyptus lanceolatus honey had darker colour as indicated by its absorbance (0.683) as compared to *Helianthus annuus* (0.612) honey. The colour of honey is an indicator of its mineral content, i.e., higher the mineral content, darker would be the colour of honey and *vice versa* (Perez-Arquillue et al. 1994). Fig 1 and 2 illustrate the effect of microwave power levels and heating duration on absorbance values of *Helianthus annuus* and *Eucalyptus lanceolatus* honey samples. Absorbance values increased with the increase in microwave levels and heating duration. Heating of honey at 280 W for 4 min revealed increases of 2.3 times in *Eucalyptus lanceolatus* and 1.15 times in *Helianthus annuus* honey samples.

Hydroxy methyl furfural content progressively increased with the increase in microwave power level and duration of

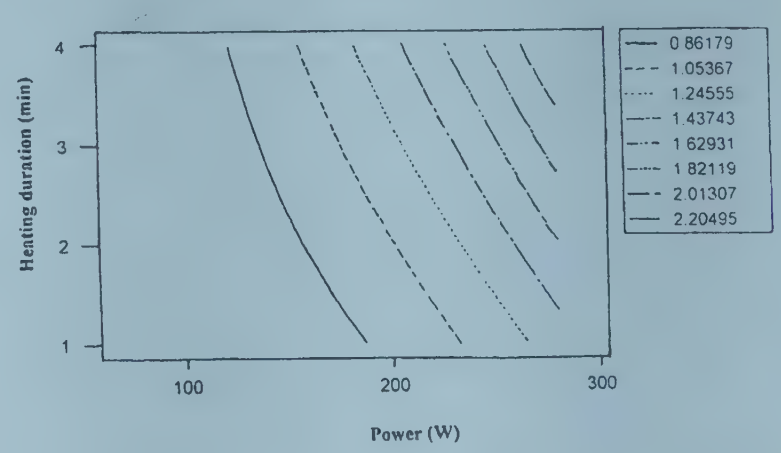

Fig. 1. Effect of microwave power levels and heating duration on absorbance value in *Eucalyptus lanceolatus*

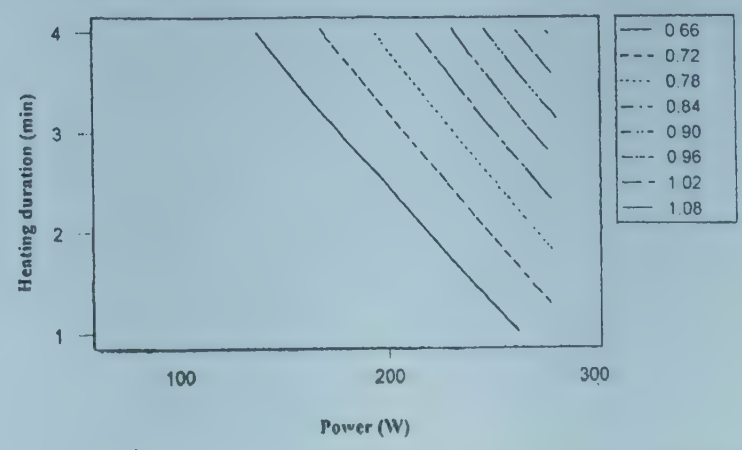

Fig. 2. Effect of microwave power levels and heating duration on absorbance value in *Helianthus annuus*

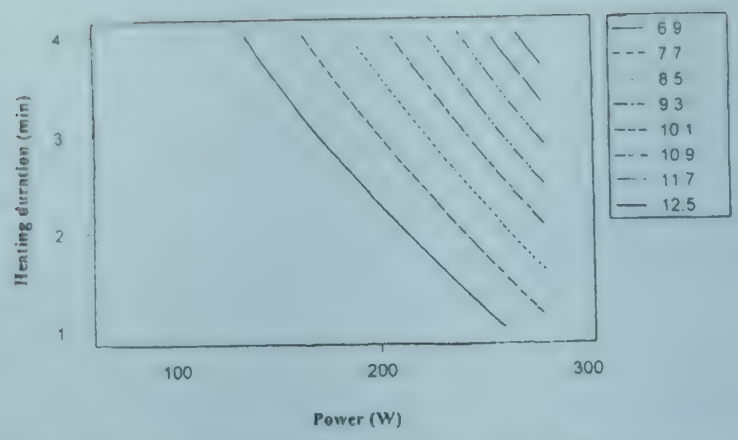

Fig. 3. Effect of microwave power levels and heating duration on absorbance value in *Eucalyptus lanceolatus*

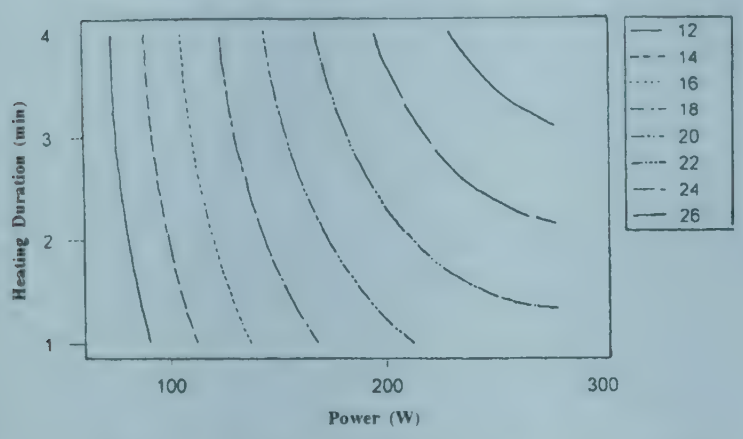

Fig. 4. Effect of microwave power levels and heating duration on HMF formation in *Helianthus annus*

heating. Fig 3-4 illustrate the effect of microwave power levels and heating duration on HMF formation in *Helianthus annuus* and *Eucalyptus lanceolatus* honey samples. Heating for 1 min at 70, 140, 210 and 280 W resulted into HMF formation of 8.32, 18.65, 19.95 and 20.15 mg/100 g, respectively, in *Helianthus annuus* in contrast to HMF content of 5.4, 6.0, 6.65 and 7.0 mg/100g, respectively in *Eucalyptus lanceolatus* honey under similar conditions. The differences in these properties may be due to differences in composition of both the honey types. The effect of composition on HMF formation in honey during heating has been reported earlier (Hase et al. 1973). The absorption of microwaves by a material has been reported to depend on its loss factor. The greater the loss factor, greater the absorption and faster is the rise in temperature (Hudiara 1998). *Helianthus annuus* honey contained more moisture (19.5%) as compared to *Eucalyptus lanceolatus* honey (16.5%). *Helianthus annuus* honey has been observed to be more ionic as compared to *Eucalyptus lanceolatus* (Bath and Singh 1999). The ionic or conductivity contribution to loss factor, ε'' can be expressed by the following equation (Buffler 1992) :

$$\varepsilon''_{\sigma} = \sigma/2\pi f\varepsilon_0 \qquad \text{---------- (1)}$$

where σ = the direct current conductivity (mhos), f = frequency in hertz and ε_0 = permitivity of free space (8.85 x 10^{-12} F/m). The equation 1 can be rewritten in the following simpler form :

$$\varepsilon''_{\sigma} = 1.80\ \sigma/f \qquad \text{---------- (2)}$$

where σ and f = mmhos/cm and Ghz (more commonly used units), respectively. Therefore, at 2.45 Ghz, system with greater conductivity contributes more to the loss factor as compared to the system with lower conductivity.

Conclusion

The effect of microwave heating on the formation of HMF and browning showed that absorbance increased with the increase in microwave power levels and duration of heating. However, microwave power levels showed a greater effect. Both the honey types differed significantly with respect to HMF formation and colour development under similar microwave heating. *Helianthus annuus* showed higher HMF formation and absorbance value as compared to *Eucalyptus lanceolatus* under similar microwave heating conditions. The models developed can be used as a tool to predict HMF and colour formation in honey during microwave heating.

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RESEARCH NOTE

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Optimization of Process Variables for Preparation of Fresh Fried Potato Chips

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Effects of frying time, temperature and thickness of potato slices on oil absorption, moisture content and colour of fresh fried chips have been studied. The loss of reducing sugars was found to have an average diffusivity of 5.01 x 10^{-9} m^2/s in case of blanching of 1.5 and 2.0 mm thick slices in boiling water. Multiple regression equations were developed for moisture, oil and colour values in the final product as a function of frying time, oil temperature and thickness of slice. For good quality potato chips, the optimum parameters were 219-221 sec frying time, 145-146°C oil temperature and 2.00 mm thickness of slice by linear programming technique.

Keywords : Diffusivity, Optimization, Frying, Potato chips, Colour development, Regression equation.

Potato chips are made by deep - fat - frying of potato slices in hot oil. Blanching of slices in hot water lowers the reducing sugars and improves the colour. Diffusivity of reducing sugar plays an important role in deciding the time of blanching. Moisture content, oil content and colour are important factors, which determine the quality of chips (Anand 1982). For longer shelf life, the moisture content of chips should be within 2 to 3% (Simpson 1969) and from the economic point of view, the oil content of chips should be as low as possible. For good appearance, the colour of chips should be light yellow. Various factors, which are responsible for final quality of chips are, tuber variety, thickness of slice, blanching time and temperature and time of frying. Keeping these in mind, the present study was undertaken to optimize the processing parameters by developing regression equations.

Potatoes purchased from local market of Kharagpur, 1993-94, had moisture content 84%, fat 0.14%, reducing sugars 0.9% on fresh weight basis. Refined groundnut oil having 1.46 refractive index was used as frying medium.

Potatoes were washed, sliced into 1.50 and 2.00 mm thicknesses and blanched in boiling water. Reducing sugar contents in the slices were measured by dinitro salicylic acid reagent method (AOAC 1965) at 20 sec intervals. Difusivity of reducing sugars (Dr) was estimated by using the equation of Crank, (1957).

$$\text{Ln}\ \frac{Xr}{Xr_0} = \text{Ln}\ \frac{8}{\Pi^2} - \frac{\Pi^2\ Dr\ \varnothing_b}{b} \qquad \ldots\ldots\ldots (1)$$

where,

Xr = reducing sugars content at time $\varnothing_b$, wt %; Xr_0 = initial reducing sugars content, wt %; b = thickness of slice, mm; $\varnothing_b$ = time of blanching, sec and Dr = diffusivity of reducing sugars, m^2/s.

The plot between ln (Xr / Xr_0) and blanching time yielded a straight line. The slope of line gave the value of diffusivity (Dr). The computed values of diffusivity were 4.89 x 10^{-9} m^2/s for 1.5 mm slices and 5.18 x 10^{-9} m^2/s for 2.00 mm slices. In order to decrease the reducing sugars content to 0.2% (Burton and Mc Weemy, 1964), the desired blanching time $\varnothing_b$ as obtained from equation (1) were 70 sec for 1.5 mm and 110 sec for 2.00 mm. The moisture content of the slices did not change during blanching.

Blanched slices (35 g) were fried in groundnut oil (1 kg) in electrically heated aluminium container at 145, 165 and 180°C. Potato slices to oil ratio of 25:1000 was chosen to give an initial temperature drop of 10°C. Slices were placed in hot oil, when temperature was 5°C higher than the set temperature maintained by a thermocouple. Potato slices were taken out at 30 sec interval and placed on tissue paper to remove the surface oil. The frying medium was changed (Sultana and Sen 1979) every 3 h of frying, during which time, its refractive index increased from 1.4623 to 1.4628. The moisture content of sample was determined by using the vacuum oven (AOAC 1984) method. Oil content was estimated in hexane in Soxhlet's apparatus for 4 h. Colour of all the fried chips was matched against red, yellow and blue colour slides of a Lovibond tintometer (Model-E) followed by arithmetic summation, C of the red, yellow and blue colour values.

Multiple regression equations were developed for each of the dependent variable, namely, moisture content M, oil content O, and colour C as a function of all variables, namely time of frying $\varnothing_f$, oil temperature T and thickness of slice b. These equations were used to find out the values of $\varnothing_f$, T and b in such a way that the sum of [M + O + ln C] becomes minimum under constraints :

$$T_1 \le T \le T_2 \qquad \ldots\ldots\ldots (2)$$

$$b_1 \le b \le b_2 \qquad \ldots\ldots\ldots (3)$$

$$M_1 \le M \le M_2 \qquad \ldots\ldots\ldots (4)$$

$$O_1 \le O \le O_2 \qquad \ldots\ldots\ldots (5)$$

$$C_1 \le C \le C_2 \qquad \ldots\ldots\ldots (6)$$

$$\varnothing_{f1} \le \varnothing_f \le \varnothing_{f2} \qquad \ldots\ldots\ldots (7)$$

The suffixes 1 and 2 used in equation (2) to (7) represented the lower and upper limits of the variables. It was decided to have the oil temperatures vary between 145 and 185°C and slice thickness between 1.5 and 2.00 mm. Therefore, in eqns. (2) and (3), T_1 = 145, T_2 = 185, b_1 = 1.5 and b_2 = 2.00. The values of $\varnothing_{f1}$, $\varnothing_{f2}$, M_1, M_2, O_1, O_2, C_1 and C_2 were fixed from

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subsequent analysis. The problem was solved with a quantitative system business (QSB) package on micro computer.

The moisture content of potato slices followed a linear relationship except the beginning and end of the cycle. The minimum frying time required to reach a final moisture content of 3% were found to be 256 sec at 145°C, 166 sec at 165°C and 111 sec at 185°C for 2.00 mm slices. The corresponding time for 1.5 mm slices were 221 sec at 145°C, 151 sec at 165°C and 99 sec at 185°C. The oil content of chips increased with increase in frying time up to 238 sec at 145°C, 150 sec at 165°C and 104 sec at 185°C for 2.00 mm slices. The corresponding time for 1.5 mm slices were 262 sec at 145°C, 170 sec at 165°C and 102 sec at 185°C. Beyond these periods, the increase in oil uptake and moisture loss were very small. The oil content of 39-41% was observed for both 1.5 mm and 2.00 mm slices, when residual moisture content was 2-3%.

From this analysis, the constraints for M (eqn. 4) and O (eqn. 5) were taken as : $M_1 = 2$, $M_2 = 3$, $O_1 = 38$ and $O_2 = 41$.

The rate constant K was estimated from a straight line plot between Ln C and frying time $\varnothing_f$. The slope of the line gave the value of rate constant (K). The colour development during deep - fat - frying of potato followed a first order reaction kinetics as indicated by linear relationship logarithm of colour with frying time for oil temperatures.

The rate constant (K) values were found to be 0.0034 at 145°C, 0.0060 at 165°C and 0.0131 at 185°C for 1.5 mm slices. The corresponding rate constant (K) for 2.00 mm slices were 0.0033 at 145°C, 0.0054 at 165°C and 0.0123 at 185°C with increase in temperature, the rate constant (K) increased. Also, the kinetic parameters from colour development did not vary much with the thickness of potato slice.

The sensory evaluation of the chips revealed that for consumer acceptance, the colour of chips should be between 7 and 9, below 7, the colour was too light and above 9, it became very dark, making them unacceptable. So, the limits of C in eqn. (6) were taken as $C_1 = 7$ and $C_2 = 9$. The amount of colour developed at the end of minimal frying time, when the moisture content is 3% is shown in Table 1. It was observed that frying time at 165°C and 185°C resulted in unacceptable colour. So, the limits of frying time (eqn. 7) were selected corresponding to an oil temperature of 145°C yielding $\varnothing_{f1}$ = 220 sec and $\varnothing_{f2}$ = 254 sec for 1.5 mm and 2.00 mm slices, respectively.

The following regression equations were used for prediction of moisture, oil and colour of chips for different time, temperature and thickness of slices.

$$M = 192.40 - 0.431\ \varnothing_f - 0.795\ T + 9.97\ b \quad (R^2 = 0.87) \quad \ldots\ldots (8)$$

TABLE 1. COLOUR VALUE AND MINIMUM TIME TO ATTAIN 3% MOISTURE IN CHIPS DURING FRYING

Oil temperature, °C	Thickness of slice 1.5 mm Colour	1.5 mm Minimum time required, sec	2.00 mm Colour	2.00 mm Minimum time required, sec
145	8.20	220	8.10	254
165	11.60	152	19.40	188
185	17.80	108	15.80	112

TABLE 2. OPTIMUM PROCESS PARAMETERS FOR DEEP - FAT - FRYING OF POTATO CHIPS

Parameters	Feasible solution I	II
Temperature of frying, T (°C)	145	146
Thickness of slice, b (mm)	2.0	2.0
Moisture content, M (Wt%)	2.0	2.0
Oil content, O (Wt%)	40.1	40.1
Colour value	8.6	8.7
Time of frying, $\varnothing_f$ (sec)	221	219

$$O = 54.99 + 0.210\ \varnothing_f + 0.399\ T - 4.90\ b \quad (R^2 = 0.88) \quad \ldots\ldots (9)$$

$$Ln\ C = -1.160 + 0.005\ \varnothing_f + 0.018\ T - 0.151\ b \quad (R^2 = 0.71) \quad \ldots\ldots (10)$$

Using the above equations and values of constraints (i.e. $T_1 = 145$, $T_2 = 185$, $b_1 = 1.5$, $b_2 = 2.00$, $M_1 = 2$, $M_2 = 3$, $O_1 = 38$, $O_2 = 41$, $C_1 = 7$, $C_2 = 9$, $\varnothing_{f1} = 220$ and $\varnothing_{f2} = 254$) in the QSB package, two feasible solutions for minimum value of (M + O + LnC) were obtained. The results shown in Table 2 revealed that, to get good quality chips (2-3% moisture, 40% oil content and 7-9 colour value), the temperature of oil should be low (145-146°C) and the frying time high (219-221 sec).

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RESEARCH NOTE

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Product Quality of Traditional Ready-to-Eat *Madeli* and Its Changes During Storage

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Madeli a traditional ready-to-eat sweet product prepared from durum wheat was analysed for its nutritional, functional, microbiological and storage qualities. The product provided 6.98 g proteins, 4.41 g fat, 1.19 g crude fibre and 2.38 g ash per 100 g. Though the keeping quality of *madeli* was 21 days in aluminium boxes and in polythene pouches, the products in polythene pouches had higher moisture, peroxide value and free fatty acids content than the products in aluminium boxes. There was significantly higher moisture and protein contents and considerable decrease *in vitro* protein and starch digestibilities and viscosities were observed in stored products.

Keywords : Durum wheat, *Madeli*, Storage quality, Ready-to-Eat sweet meat.

The trend in consumption of convenience foods is increasing due to the increasing number of working women population concomitant with the increase in per capita income, urbanisation, scarcity of household labour, lack of time and hectic schedules, compelling the consumer to look for foods of convenience, easy commercial availability, culturally acceptable, nutritive and minimally processed with longer shelf life. The tendency of the quality conscious urban rich is to go in for wheat-based processed food products (Nagarajan 1997). Among the wheat species, durum wheat is known for its pasta making quality and superior semolina yield. Several South Indian snacks like *uppuma, kesari bath, sajjaka, rava idli* and *holige* are prepared from durum wheat. Many of value-added products viz., vermicelli, noodles and macaroni products have entered the market and are also becoming popular. An attempt was made to evaluate the quality of *madeli,* a traditional ready-to-eat product prepared from durum, an ideal wheat for producing coarse flour and the changes in quality characteristics of the product during storage were also studied.

Preparation of Madeli : *Madeli* is a traditional ready-to-eat soft textured granular sweet product prepared from baked *chapati* powder, containing wheat and *Bengal*gram *dhal* and mixed with jaggery. It is used as a special dish during *Makar Sankranti* (harvest) festival.

Durum wheat was procured from Wheat Improvement Project, UAS, Dharwad. *Bengal*gram *dhal* and jaggery were purchased from the local market. *Madeli* was prepared by standardised method as described by Pattan (1999). Wheat and *Bengal*gram *dhal* were mixed in 80:20 proportion, milled into coarse flour of particle size 0.2 - 0.4 mm, made into a stiff dough by adding required amount of water, rolled into thick *chapati* of 4 mm and diameter of 13 cm, baked at 127°C for 3-4 min. *Chapati* was broken into pieces immediately and milled into powder of particle size 0.4-0.6 mm. Jaggery was added to this powder in 60:40 proportion.

Proximate composition of *madeli* was analysed using standard AOAC (1984) procedures. *In vitro* protein and starch digestibilities were estimated using suitable enzymatic method (Mouliswar et al. 1993). Swelling power and solubility of the product were analysed by the procedure of Schoch (1964). Cold and hot paste viscosities of *madeli* were tested at different concentrations using viscometer (Brandtzaeg et al. 1981) at 100 rpm with spindle no 2 (RVT Model, Brookefield Viscometer, USA). Microbiological quality of the product was assessed in terms of total bacterial counts (TBC) as per the procedure described by Lakshmanan et al (1971).

The storage stability was observed by storing the product in aluminium box, a conventional storing unit and compared the product quality with that stored in polythene pouch (The water vapour transmission rate at 38°C, 90% RH was 3.3) at ambient conditions. Stored samples were analysed at weekly intervals for moisture, peroxide value and free fatty acids as per standard procedure (AACS 1983), till the product was acceptable organoleptically. Further, the end product was observed for changes in proximate composition, *in vitro* protein and starch digestibilities as per procedures given earlier.

Data on nutritional, functional and microbiological qualities of fresh *madeli* and of stored samples in different packages at room temperature, presented in Table 1 reveal that, in fresh condition, the product provided 6.98% proteins, 4.41% fat, 2.38% ash and 1.19% crude fibre. Total carbohydrates obtained by difference was 85.04% in the product. *Madeli* had 89.01% *in vitro* protein digestibility, while *in vitro* starch digestibility was 61.80 mg/100 mg. It was also observed that the swelling power values of *madeli* were 2.60 and 5.55 g/g, respectively at room and boiling temperatures. The cold paste viscosities estimated at 10 and 20% slurry concentration were 21.67 and 40.00 Cp units, respectively. The total bacterial count (TBC) in fresh *madeli* was 1.67 x 10^4 cfu/g of sample. Significantly higher moisture and protein contents were observed in stored *madeli* as compared to fresh product. Among the products in different packages, *madeli* in polythene pouch had higher moisture content than samples in aluminium box. From the results, it was also observed that there were slight decrease in crude fibre and fluctuations in the values of fat and ash contents. Fluctuations in protein content were also observed in ready-to-eat products at 3 months of storage (Uma and Meera Rao 1999). The increased TBC in stored *madeli* was contributed in increased

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TABLE 1. NUTRITIONAL, FUNCTIONAL AND MICROBIOLOGICAL QUALITIES OF TRADITIONAL READY-TO-EAT FRESH AND STORED *MADELI* SAMPLES IN DIFFERENT PACKAGES

Parameters	Fresh sample	Stored sample (21 days) Aluminium box	Polythene pouch
Nutritional quality			
Proximate composition*			
Moisture, %	13.26	17.57	18.07
Proteins, %	6.98	7.20	7.11
Fat, %	4.41	4.34	4.39
Ash, %	2.38	2.41	2.44
Crude fibre, %	1.19	1.14	1.09
Total carbohydrates by difference, %	85.04	84.91	84.97
***In vitro* digestibilities**			
Proteins, %	89.01	86.68	73.74
Starch, mg glucose/100 mg sample	61.80	58.06	56.18
Functional quality			
Swelling power, g/g			
Room temperature	2.60	ND	ND
Boiling temperature (100°C)	5.55	ND	ND
Percent solubility			
Room temperature	4.40	ND	ND
Boiling temperature (100°C)	33.18	ND	ND
Viscosity, Cp units			
Cold paste, 10%	21.67	20.00	16.66
Cold paste, 20%	40.00	35.00	30.00
Hot paste, 10%	70.33	64.66	65.66
Hot paste, 20%	310.00	289.33	295.00
Microbiological quality			
Total bacterial count, cfu/g	1.67×10^4	3.67×10^4	4.33×10^4

ND Not Determined
* On dry weight basis

protein content in the sample. Drastic reductions in *in vitro* protein and starch digestibilities were observed in stored samples. However, reduction was higher in samples stored in polythene pouches. Reduced *in vitro* digestibilities may be due to formation of complexes between carbohydrates and proteins and other substances present in grain itself, thereby reducing accessibility of carbohydrates and protein to enzyme action (Adsule 1985). The reduced viscosity in stored *madeli* was observed and this might be due to the biochemical changes, that take place during storage (Fisher et al. 1987). Bell et al (1979) observed that the changes in rheological properties in stored flours were mainly due to the increased lipolyse activity. Though there was increase in microbial load from 1.67×10^4 to 4.33×10^4, the increase was within the ISI specification of TBC for RTE weaning foods (Anon 1969). Higher TBC in samples in polythene pouches may be due to higher permeability of polythene pouch to air and water, which might have enhanced the bacterial growth.

Storage stability of *Madeli* packed in different packages, at ambient conditions (Fig. 1) revealed that, the samples packed in polythene pouches had the higher mean moisture content (16.28%) than those in aluminium boxes (15.76%) and also had the higher mean peroxide value (4.73 meq/kg fat) and free fatty acid content (0.227%) compared to sample in aluminium box, during the storage period of 21 days. Diffusion of gases and vapour through microscopic holes or by activated diffusion of polythene pouches might have contributed to increased moisture content during storage (Palling 1980). Higher peroxide value and free fatty acids in the samples of polythene pouches could be due to higher oxygen transmission rate and exposure of

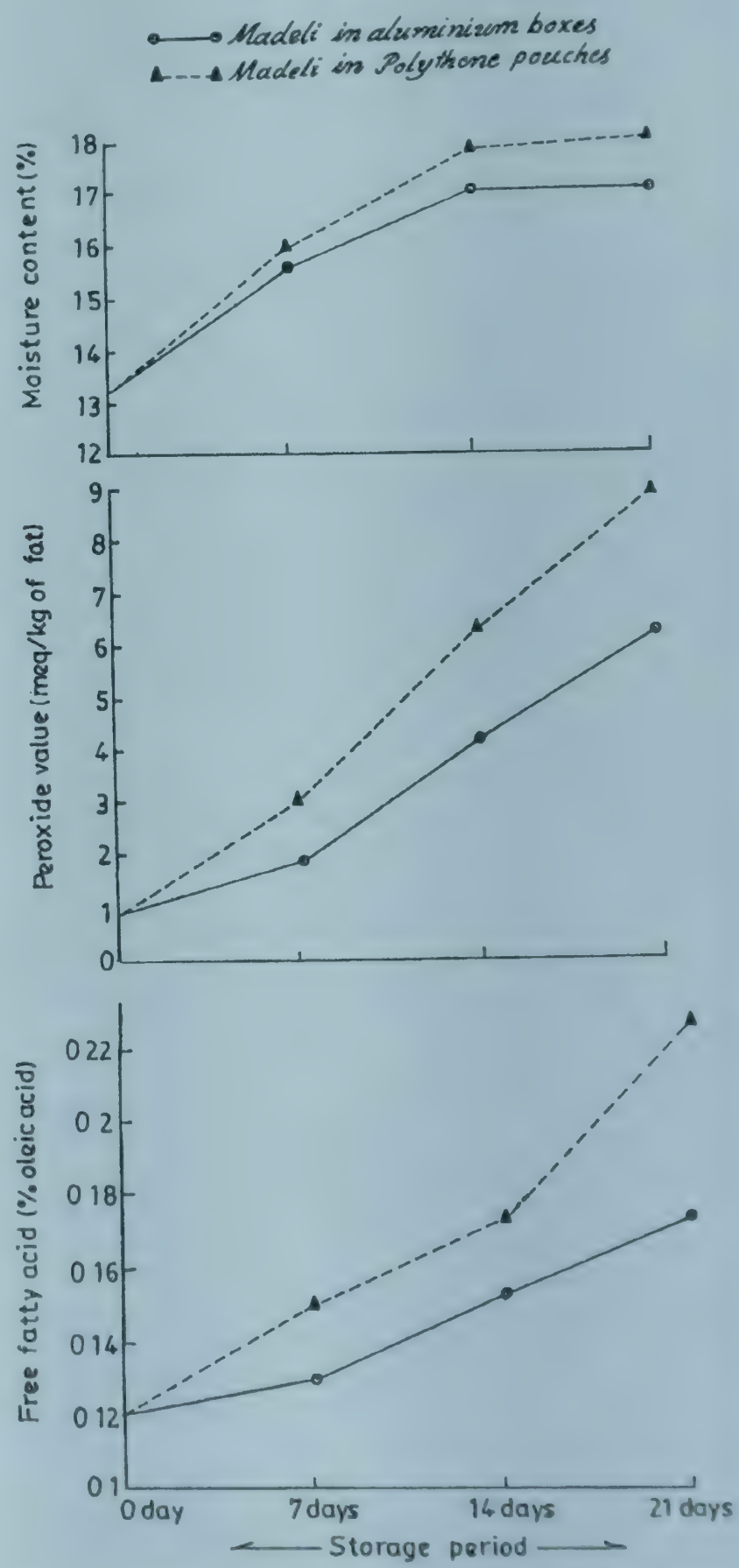

Fig. 1. Changes in moisture, peroxide value and free fatty acids of *madeli* in different packages during storage at room temperature

TABLE 2. CHANGES IN ORGANOLEPTIC QUALITY OF *MADELI* IN DIFFERENT PACKAGES DURING STORAGE AT ROOM TEMPERATURE

Organoleptic qualities	Storage, days							
	Aluminium box				Polythene pouch			
	0	7	14	21	0	7	14	21
Appearance	4.75	4.10	3.70	3.10	4.75	4.10	3.20	2.70
Colour	4.48	4.30	3.40	3.10	4.48	4.20	3.20	3.10
Texture	4.52	4.60	4.60	3.20	4.52	4.50	4.60	3.10
Taste	4.62	4.40	3.60	2.50	4.62	4.10	3.40	2.00
Flavour	4.80	4.20	3.00	4.30	4.80	4.20	3.10	2.00
Overall acceptability	4.80	4.00	3.30	1.80	4.80	3.60	2.30	1.30

CD Values

	Appearance	Colour	Texture	Taste	Flavour	Overall acceptability
Packages (P)	0.337	NS	NS	0.321	NS	0.482
Duration (D)	0.229	0.235	0.264	0.416	0.375	0.284
P X D	0.234	NS	NS	0.352	NS	0.378

foods in transparent pouches, thereby increasing production of degradation compounds (Palling 1980).

Changes in organoleptic quality of *madeli* stored in different packages during storage at room temperature, was evaluated by trained panelists. Table 2 reveals that the organoleptic scores decreased significantly within a period of 21 days. The samples were in acceptable condition at the end of 21 days. Though the samples stored in polythene pouches had significantly lower scores than in aluminium box, the product had acceptable scores of more than 1.00 at 21 days. The product might have reached unacceptable condition earlier in polythene pouches within a week of next analysis. However, both the stored samples were analysed at a week's interval and found unacceptable.

It may be concluded that *madeli* a traditional ready-to-eat sweet product prepared from durum wheat has a shelf life of 21 days. It can be exploited commercially as a convenient reay-to-eat soft food in supplementary feeding programmes, old age homes and in local markets as a food for occasions.

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Effect of Soaking, Germination and Cooking on Phytic Acid and Hydrochloric Acid Extractability of a Pigeonpea Cultivar

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Early maturing and high yielding cultivar of pigeonpea 'ICPL-151' released by ICRISAT (International Crop Research Institute for Semi-Arid Tropics) Centre, Hisar contained significant amount of phytic acid i.e., 869 mg per 100 g. When this cultivar was subjected to various domestic processing and cooking methods viz., soaking (6, 12, 18 h), soaking (12 h) and dehulling, ordinary as well as pressure cooking and germination (24, 36, 48 h), a considerable decrease in the level of phytic acid and remarkable increases in HCl-extractability of calcium, iron and phosphorus were noticed. Maximum decrease in phytate content with subsequent increase in non-phytate phosphorus was brought about by germination, followed by pressure cooking of soaked and dehulled seeds and ordinary cooking of soaked and dehulled seeds. The total calcium, phosphorus and iron contents of pigeonpea were 210.6, 437.2 and 10.88 mg/100 g, respectively, which were lowered to varying extents upon soaking and dehulling. HCl-extractability of calcium and phosphorus was the highest in sprouted (48 h) seeds, whereas that of iron was maximum in soaked and dehulled seeds, subjected to pressure cooking. The improvement in HCl-extractability may be due to reduction in phytate level. A significant ($P<0.05$) correlation found between phytic acid and HCl-extractability of minerals strengthened the present findings.

Keywords : Pigeonpea, Phytic acid, HCl-extractability, Calcium, Phosphorus, Iron.

Pigeonpea, like other legumes has an important place in the Indian diets. Pigeonpea contains low concentration of fat, moderate amount of fibre, good amount of proteins and a reasonable balanced range of all dietary essential minerals. Besides these nutrients, this legume is also rich in antinutritional factors viz., phytic acid, polyphenols, saponins and trypsin inhibitors etc., which are known to limit the utilization of legumes for human nutrition. Phytic acid binds with the divalent cations and lowers their bioavailability. Hence, it is imperative to reduce the contents of this antinutrient by any of the processing methods for effective utilization of pigeonpea.

In Indian households, simple processing and cooking methods followed for legumes include soaking, dehulling, ordinary cooking, pressure cooking and germination. These processing and cooking methods may affect the phytic acid content, which may consequently improve the bioavailability of minerals. This information for pigeon peas is lacking at present. Hence, the present communication deals with the effect of various domestic processing and cooking methods on phytic acid and HCl-extractability of minerals. HCl-extractability of minerals in foods is an index of their bioavailability from the foods (Chompreeda and Fields 1984). Thus, the solubility of minerals in food, subject to *in vitro* gastric intestinal digestion, is an useful indicator of minerals bioavailability (Kim and Zemel 1986).

Materials : The seeds of 'ICPL-151', which is a variety of pigeonpea *(Cajanus cajan)* was procured from the ICRISAT (International Crop Research Institute for Semi-Arid Tropics) Centre, Hisar, India. The selection of this variety was done on the basis of high yield and early maturity. The seeds were cleaned of dust, cracked and broken seeds and other foreign material prior to domestic processing and cooking treatments. Raw seeds were powdered (0.5 mm sieve) in an electric grinder (Cyclotec, M/S Tecator, Hoganas, Sweden), packed in air tight containers and were used as control. The processing and cooking treatments were done in triplicate.

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Processing and cooking methods

Soaking and dehulling : Seeds were soaked in double distilled water for 6, 12 and 18 h at 30°C. Seed to water ratio was 1:5 w/v. The soaked seeds were washed and rinsed with double distilled water. After soaking the seeds overnight (12 h), hulls were removed manually.

Ordinary cooking : The unsoaked, soaked (12 h) and soaked (12 h) and dehulled seeds were cooked in crude fibre beakers. The amount of water used for ordinary cooking was three times the weight of the seeds. Seeds were cooked, until they became soft as felt between the fingers.

Pressure cooking : Pressure cooking of unsoaked, soaked (12 h) and soaked (12 h) and dehulled seeds was done at 1.5 kg/cm^2. Water used for pressure cooking was twice the weight of seeds.

Germination : The soaked seeds (12 h) were washed and rinsed with distilled water. The seeds were rolled in germination paper and kept in an incubator at 30°C for 24, 36 and 48 h.

Preparation of processed samples : All the processed seeds were dried in a hot air oven kept at 60°C to a constant weight, ground in an electric grinder (Cyclotec, M/s Tecator, Höganäs, Sweden) using 0.5 mm sieve size and packed in air tight containers for further chemical analysis.

Chemical analysis : Phytic acid content was determined by the method of Davies and Reid (1979). For analysing total minerals, the control and processed legume samples were wet acid digested with diacid i.e., a nitric acid and perchloric acid mixture (HNO_3:$HClO_4$::5:1, v/v) in the digestion chamber. The digested samples were dissolved in double distilled water and filtered (Whatman No 42). The filtrate was made up to 50 ml with double distilled water and used for the determination of total calcium, phosphorus and iron contents. Calcium in the digested sample was determined by titration method by Vogel (1962). Phosphorus was determined colorimetrically by the method of Chen et al (1956). Phytate phosphorus was derived by using the following formula (Reddy et al. 1982).

$$\text{Phytate phosphorus, mg} = \frac{A \times 28.18}{100}$$

where A = the phytate content, mg

Non-phytate phosphorus was calculated as a difference between the total phosphorus and phytate phosphorus.

Iron in acid-digested samples was determined by using an Atomic Absorption Spectrophotometer, 2380, Perkin-Elmer (USA) according to the method of Lindsey and Norwell (1969).

For HCl-extractability of minerals, the samples were extracted with 0.03 N HCl by shaking the contents at 37°C for 3 h. The clear extract obtained after filtration with Whatman No 42 filter paper was oven-dried at 100°C and wet acid digested. The amounts of the HCl-extractable calcium, phosphorus and iron in the digested samples were determined by the methods as described above for estimation of total minerals :

$$\text{Mineral extractability, \%} = \frac{\text{Mineral extractability in 0.03 N HCl}}{\text{Total minerals}} \times 100$$

Statistical analysis : The data were subjected to statistical analysis of variance in a complete randomized design and correlation coefficients were derived according to standard method (Panse and Sukhatme 1961).

Phytic acid : The content of phytic acid known to be the major storage form of phosphorus in pigeonpea and considered to be an antinutritional factor was 869 mg/100 g, (Table 1). Soaking as well as dehulling of the soaked seeds contributed significantly ($P < 0.05$) in reducing the phytic acid content. On 6 h soaking, the per cent loss was 6. With an increase in the period of soaking i.e., 6 to 12 and 12 to 18 h, further reduction in phytate content of pigeonpea was observed. After 18 h of soaking, the loss was 26 %, indicating that longer the period of soaking, more were the losses in phytate content (Table 1). The cumulative effect of soaking and dehulling was more pronounced than that of soaking alone for lowering the phytate content.

The losses in phytates during soaking of pigeon pea may be because of leaching out of phytate ions into soaking water under the influence of concentration gradient (difference in chemical potential), which governs the rate of diffusion. Similar results for reduction in phytic acid in the soaked as well as soaked and dehulled legumes have been reported earlier (Deshpande and Cheryan 1983; Ologhobo and Fetuga 1984; Khokhar and Chauhan 1986 a, b; Kataria et al. 1988; 1989; Duhan et al. 1989; Kaur and Kapoor 1990; Bishnoi et al. 1994; Jood et al. 1998).

Ordinary cooking of unsoaked, soaked as well as soaked and dehulled pigeonpea seeds brought about a significant decrease in phytic acid content, when compared to control (Table 1). A reduction in phytic acid content was noticed after ordinary cooking of unsoaked seeds but this loss appeared to be less than that in seeds, which were cooked after soaking or soaking and dehulling. The per cent loss in phytic acid content of soaked and cooked seeds was 15 and it was more than double, when the seeds were soaked, dehulled and then cooked.

TABLE 1. EFFECT OF DOMESTIC PROCESSING AND COOKING METHODS ON PHYTIC ACID, PHYTATE PHOSPHORUS AND PERCENTAGE OF TOTAL PHOSPHORUS IN PIGEONPEA (ON DRY MATTER BASIS)

Treatment	Phytic acid, mg/100 g	Total phosphorus, %	Phytate phosphorus		Non-phytate phosphorus, %	Phosphorus extractability, %
			Total, mg	% of Total, %		
Control	869 ± 7	437.2 ± 0.7	244.8 ± 0.9	56.01 ± 0.03	43.99 ± 0.03	31.6 ± 0.8
Soaking						
6 h	821 ± 7 (–6)	424.3 ± 0.1 (–3)	231.35 ± 0.8	54.52 ± 0.42	45.48 ± 0.42	32.8 ± 1.0 (+4)
12 h	770 ± 8 (–11)	414.3 ± 0.1 (–5)	216.98 ± 0.8	52.37 ± 0.37	47.63 ± 0.37	33.8 ± 1.0 (+7)
18 h	646 ± 15 (–26)	399.6 ± 0.8 (–9)	182.04 ± 0.4	45.55 ± 0.30	54.45 ± 0.30	34.6 ± 0.8 (+9)
Soaking and dehulling	630 ± 6 (–28)	384.1 ± 0.5 (–12)	177.53 ± 0.5	46.21 ± 0.57	53.79 ± 0.57	36.3 ± 0.1 (+15)
Ordinary cooking						
Unsoaked	832 ± 7 (–4)	437.2 ± 0.7	234.45 ± 0.6	56.01 ± 0.50	43.99 ± 0.50	35.2 ± 0.2 (+11)
Soaked	740 ± 5 (–15)	414.3 ± 0.1 (–5)	208.53 ± 0.5	56.58 ± 0.42	43.42 ± 0.42	38.3 ± 0.2 (+21)
Soaked and dehulled	600 ± 6 (–31)	384.1 ± 0.5 (–12)	169.08 ± 0.4	54.29 ± 0.30	45.71 ± 0.30	43.3 ± 0.2 (+37)
Pressure cooking						
Unsoaked	760 ± 18 (–13)	437.2 ± 0.7	214.16 ± 0.8	48.98 ± 0.25	51.02 ± 0.25	37.6 ± 0.8 (+19)
Soaked	720 ± 16 (–17)	414.3 ± 0.1 (–5)	202.89 ± 0.5	48.97 ± 0.30	51.03 ± 0.30	40.6 ± 0.8 (+28)
Soaked and dehulled	575 ± 17 (–34)	384.1 ± 0.5 (–12)	162.03 ± 0.4	42.18 ± 0.37	57.82 ± 0.37	47.3 ± 0.2 (+50)
Germination						
24 h	595 ± 9 (–32)	414.3 ± 0.1 (–5)	167.67 ± 0.4	40.47 ± 0.20	59.53 ± 0.20	40.6 ± 0.8 (+28)
36 h	575 ± 7 (–34)	414.3 ± 0.1 (–5)	162.03 ± 0.4	39.10 ± 0.21	60.90 ± 0.21	47.3 ± 0.2 (+50)
48 h	560 ± 7 (–36)	414.3 ± 0.1 (–5)	157.80 ± 0.4	38.08 ± 0.11	61.92 ± 0.11	61.6 ± 0.8 (+95)
SE (m)	± 4.34	± 4.38	± 0.39	± 0.59	± 0.09	± 0.23
CD ($P<0.05$)	13.09	13.49	1.21	1.81	0.29	0.70

Values are mean ± SD of three independent determinations
Figures in parentheses indicate per cent decrease (–) or increase (+) over control values

On pressure cooking of unsoaked seeds, there was a significant ($P<0.05$) reduction in phytic acid content i.e., 13% (Table 1). Pressure cooking of soaked as well as soaked and dehulled seeds caused a greater loss than by pressure cooking of unsoaked ones. Phytic acid content was reduced by 13, 17 and 34%, when unsoaked, soaked and soaked and dehulled seeds were pressure cooked, respectively.

According to deBoland et al (1975), the differences in the losses of phytic acid contents during cooking probably be explained on the basis that phytase activity at a temperature of 40 to 55°C may degrade inositol hexaphosphate to pentaphosphate or lower molecular weight forms. Kumar et al (1978) observed that phytic acid content decreased because insoluble complexes between phytate and other components were formed during cooking.

Germination too resulted in a significant ($P<0.05$) loss of phytic acid. As the period of germination was prolonged i.e., 0 to 24, 24 to 36 and 36 to 48 h, a significant ($P<0.05$) and successive reduction in phytic acid was noticed (Table 1). A loss of 32 % occurred during 24 h germination, which further enhanced with an increase in the period of germination. After 48 h germination, upto 36% loss was noticed. Loss of phytic acid during germination may be due to hydrolytic activity of phytase reported to be present in various plant foods including legumes (Mandal et al. 1972; Lolas and Markakis 1975).

Phytate, non-phytate and extractable phosphorus : Fifty six per cent of the total phosphorus was present as phytate phosphorus in the unprocessed pigeonpea seeds. Various processing and cooking methods resulted in significant decreases with corresponding remarkable increases in non-phytate phosphorus and HCl-extractable phosphorus (Table 1). Germination was found to be the best processing method, followed by pressure cooking and ordinary cooking of soaked and dehulled seeds for reducing the content of phytate phosphorus and improving the HCl-extractability of phosphorus. Cleavage of phosphorus from phytic acid may explain the increased level of non-phytate phosphorus and higher HCl-extractability of phosphorus in the processed and cooked pigeonpea seeds.

Total minerals : The total calcium, phosphorus and iron contents of pigeonpea cultivar were 210.6, 437.2 and 10.88 mg/100 g, respectively (Tables 1 and 2). With an increase in the period of soaking, losses in the contents of total calcium, phosphorus and iron were observed. Dehulling of soaked seeds further led to a significant reduction in all the three minerals studied. The losses in the mineral contents of soaking may be attributed to leaching out of these minerals into the soaking water (Kumar et al. 1978). Dehulling is a contributing factor for the losses of total mineral contents, as minerals present in the hulls might have been lost during dehulling.

Cooking of unsoaked seeds caused no loss, whereas a significant decrease was noticed, when the soaked and dehulled seeds were cooked. No loss of minerals occurred during cooking but some loss, which occurred was due to the fact that seeds

TABLE 2. EFFECT OF DOMESTIC PROCESSING AND COOKING METHODS ON TOTAL MINERAL CONTENTS AND THEIR EXTRACTABILITY IN PIGEONPEA (ON DRY MATTER BASIS)

Treatment	Minerals			
	Calcium		Iron	
	Total, mg/100 g	Extractability, %	Total, mg/100 g	Extractability, %
Control	210.06 ± 0.8	44.2 ± 0.94	10.88 ± 1.12	35.20 ± 0.01
Soaking				
6 h	200.2 ± 0.5 (–5)	44.9 ± 0.61 (+2)	10.65 ± 0.81 (–2)	36.3 ± 0.12 (+3)
12 h	195.1 ± 0.2 (–7)	45.8 ± 0.60 (+4)	10.47 ± 0.68 (–4)	39.6 ± 0.32 (+13)
18 h	190.2 ± 0.3 (–10)	46.9 ± 0.52 (+6)	10.47 ± 0.81 (–4)	41.5 ± 0.13 (+18)
Soaking and dehulling	165.6 ± 0.8 (–21)	54.1 ± 0.92 (+22)	9.96 ± 0.04 (–8)	43.8 ± 0.30 (+24)
Ordinary cooking				
Unsoaked	210.6 ± 0.8	48.9 ± 0.61 (+11)	10.88 ± 1.12	42.4 ± 0.37 (+20)
Soaked	195.1 ± 0.2 (–7)	50.3 ± 0.94 (+14)	10.35 ± 1.12 (–5)	45.5 ± 0.40 (+29)
Soaked and dehulled	165.6 ± 0.8 (–21)	52.3 ± 0.10 (+18)	10.15 ± 1.12 (–7)	47.8 ± 0.42 (+36)
Pressure cooking				
Unsoaked	210.6 ± 0.8	49.3 ± 0.61 (+12)	10.88 ± 1.12	47.4 ± 0.03 (+35)
Soaked	195.1 ± 1.0 (–7)	50.2 ± 0.61 (+14)	10.35 ± 1.12 (–5)	50.7 ± 0.06 (34)
Soaked and dehulled	165.6 ± 0.8 (–21)	56.3 ± 0.61 (+27)	10.15 ± 1.12 (–7)	49.8 ± 0.20 (+41)
Germination				
24 h	195.1 ± 0.2 (–7)	58.2 ± 0.5 (+32)	10.35 ± 1.12 (–5)	44.5 ± 0.37 (+26)
36 h	195.1 ± 0.2 (–7)	62.2 ± 0.5 (+41)	10.35 ± 1.12 (–5)	46.4 ± 0.42 (+32)
48 h	195.1 ± 0.2 (–7)	64.2 ± 0.5 (+45)	10.35 ± 1.12 (–5)	48.3 ± 0.40 (+37)
SE(m)	± 0.36	± 0.48	± 0.11	± 0.15
CD ($P<0.05$)	1.08	1.43	0.32	0 45

Values are mean ± SD of three independent determinations
Figures in parentheses indicate per cent decrease (–) or increase (+) over control values

prior to cooking were soaked for 12 h and further dehulled. In the present study, as expected, maximum loss in calcium has been reported by dehulling. The reason being that calcium has been reported to be found mainly in the testa (61.4%), whereas only 26.8% and 11.8% were present in the embryo and cotyledon, respectively (Singh et al. 1968). Germination itself, too had no significant effect upon loss of total calcium, phosphorus and iron. It can be concluded that the loss was not due to germination, but again it was due to the soaking (12 h) done prior to sprouting.

Extractability of minerals : Various domestic processing and cooking methods had significant ($P<0.05$) enhancing effect on the HCl-extractability of dietary minerals including calcium, phosphorus and iron. As a result of soaking, dehulling, ordinary cooking, pressure cooking and germination, HCl-extractability in 0.03 N HCl, which is an index of the bioavailability of the minerals, improved to varying extents.

On 6 h soaking, the extractability of calcium, phosphorus and iron improved to 2, 4 and 3 % (Tables 1 and 2) As the period of soaking was increased from 6 to 12 and 12 to 18, a successive improvement in calcium, phosphorus and iron extractability was observed. After 18 h soaking, 2 to 3-fold increase was noticed in various minerals. Soaking followed by dehulling, further caused an increase in the HCl-extractability of minerals.

Ordinary cooking as well as pressure cooking could enhance the extractability of minerals of unsoaked pigeonpea seeds but it was obviously less than that of soaked as well as soaked and dehulled seeds. Moist heating i.e., pressure cooking could improve the extractability of pigeonpea seeds to a greater extent than ordinary cooking (Tables 1 and 2).

Increase in the germination period significantly ($P<0.05$) affected the extractability of calcium, phosphorus and iron. After 48 h germination, extractabilities of calcium and phosphorus were found to be maximum, when compared to those in rest of the unprocessed and cooked pigeonpea seeds. Pressure cooking of soaked-dehulled seeds brought about maximum improvement in iron extractability, followed by germination (48 h), pressure cooking of soaked and unsoaked seeds, ordinary cooking of soaking-dehulled seeds, germination for 36 h, ordinary cooking of soaked seeds, germination for 24 h, and soaking alone in descending order. Some results have also been reported earlier on this aspect (Bishnoi and Khetarpaul 1995; 1996 a, b, 1997).

As the divalent cations including calcium, iron etc. are generally present in association with phytic acid in plant foods, it may be responsible for the lower extractabilities of these divalent cations. Decrease in the level of phytic acid by various domestic and processing methods viz., soaking, dehulling, ordinary cooking, pressure cooking and germination has been reported by previous workers (Kaur and Kapoor 1990; Bishnoi et al. 1994; Jood et al. 1998) and in this study too, adoption of these methods may possibly have released these metallic ions in free form for their increased HCl-extractability. The antinutrients i.e., phytic acid in the processed pigeonpea was found to have a negative correlation with extractable calcium, phosphorus and iron (Table 3), which ascertained the role of phytic acid in lowering the extractabilities of divalent cations in plant foods.

TABLE 3. CORRELATION COEFFICIENT OF PHYTIC ACID WITH HCl-EXTRACTABILITY OF CALCIUM, PHOSPHORUS AND IRON

Treatment	HCl-extractability of minerals		
	Calcium	Phosphorus	Iron
6 h soaking	–0.2312	–0.2930	–0.3940
12 h soaking	–0.2930	–0.3240	–0.4251*
18 h soaking	–0.3137	–0.3347	–0.4359*
Soaked (12 h), dehulled	–0.3296	–0.4183*	–0.5190*
Unsoaked and cooked	–0.4178*	–0.4919*	–0.5923**
Soaked (12 h) and cooked	–0.4903	–0.5459**	–0.6462**
Soaked (12 h), dehulled and cooked	–0.5455**	–0.4310*	–0.5330*
Unsoaked and pressure cooked	–0.4296*	–0.5213**	–0.6223**
Soaked (12 h) and pressure cooked	–0.5183**	–0.6497**	–0.7470**
Soaked (12 h), dehulled and pressure cooked	–0.6457**	–0.6614**	–0.7634**
24 h germination	–0.4923*	–0.5463**	–0.6465**
36 h germination	–0.5190**	–0.6527**	–0.7539**
48 h germination	–0.6517**	–0.6724**	–0.7726**

* Significant at 5% level
** Significant at 1% level

All the different processing and cooking methods were found to be beneficial for lowering the phytic acid content and improving the bioavailability of dietary essential minerals. Out of all, germination was found to be the best method, followed by pressure and ordinary cooking in this regard. Hence, for proper utilization of legumes especially in developing countries, these processing and cooking methods should be followed, as they not only save time, energy and fuel consumption but also enhance the nutritional quality by lowering the content of antinutrients and increasing the bioavailability of minerals.

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RESEARCH NOTE

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Influence of Storage Conditions on the Survival Period of the Clam *Meretrix casta*

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The effects of temperature, wet/dry packing and oxygen on the survival of live clams (*Meretrix casta*), were studied. The survival period was longest in the temperature range of 10-20°C. Packing clams in polyethylene bags with water and oxygen had little effect on the extension of the survival period. An insulated box was fabricated and clams were chill-stored at varying temperatures from 11 to 22°C. Clams survived for a period of 7-8 days when chill-stored, whereas these survived only for a period of 3-4 days at room temperature.

Keywords : Clams, Live transport, Packing.

Shallow water near the coast or esturies provide good environmental conditions for the growth of the clam, *Meretrix casta*. By virtue of their filter feeding system, shell fish accumulate pathogenic bacteria, when grown in polluted water (Nambudiri et al. 1995). Clams are required to be brought alive to the processing plant, since they have necessarily to be depurated before being shucked (Balachandran and Surendran 1985). Attempts have been made to extend the survival period of bivalves during transit by altering the conditions of storage (Otwell et al. 1986; Prochazha and Griffith 1991; Mohamed and Devaraj 1997; Srinivasa Gopal et al. 1985). The present investigation was aimed at developing a viable technology for extending the survival period of the commercially important clam, *Meretrix casta.*

Live clams of average size 2.5 cm, were collected off the Vypeen coast. A preliminary test was conducted in order to determine the temperature at which survival period of the clams was maximum. Clams were packed in 200 gauge polyethylene bags (30 x 23 cm) at the rate of 25 per bag. Clean water, collected from the area of catching, was added to each bag, sufficient to submerge all clams (wet pack). The bags were sealed using rubber bands, divided into various lots and each lot was placed in a BOD incubator maintained at a particular temperature, ranging from 5 to 25°C with one lot at room temperature to serve as the control.

For further studies, clams were packed as wet pack or as dry pack (without addition of water). One lot from each packing was filled with oxygen at the rate of about 500 ml per bag. All the bags were sealed and stored at 15°C in a BOD incubator. Controls, maintained at room temperature were provided for each treatment.

In another set of experiment, an insulated box was fabricated using an empty tea chest of dimension 40 x 40 x 40 cm. It was given an inner lining with thermocole slates of thickness 1.5 cm and density 14 kg/m^3, wrapped in polyethylene film. A perforated aluminium support was placed at the bottom the box. Ice in 1:1 proportion by weight of clams was packed in a polyethylene bag and placed below the support. Bags of clams, packed as in the previous trial were placed on the support to a height of about 20 cm. The temperature at the bottom layer of the clams and near the top layer was measured by a freezer temperature monitor. The box was closed with a thermocole-lined lid. Re-icing was carried out every day. Controls were set up as for the previous trials.

For all trials, sampling was done every day, for which two bags were drawn from each lot and checked for the survival of the bivalves. Clams that resisted any attempt to open the shell using a scalpel, were considered alive and healthy.

In the preliminary trial, the maximum survival period obtained was 8-9 days at temperatures ranging from 10 to 20°C (Table 1). Based on this, a temperature of 15°C was selected for further studies. The clams consistently showed marked increases in the duration of survival at 15°C compared to those at room temperature in all the combinations of storage conditions (Table 2). However, addition of water and packing under oxygen seemed to have little effect on the

TABLE 1. EFFECT OF TEMPERATURE ON THE SURVIVAL OF THE CLAM, *MERETRIX CASTA*, DURING STORAGE (PERCENTAGE SURVIVAL)

Storage period, days	Temperature, °C					
	5	10	15	20	25	Room temperature*
1	100	100	100	100	100	100
2	100	100	100	100	100	100
3	92	100	100	100	100	92
4	92	100	100	100	68	72
5	0	100	100	100	0	0
6	-	100	100	100	-	-
7	-	100	92	100	-	-
8	-	100	92	76	-	-
9	-	84	84	60	-	-
10	-	0	0	0	-	-

*Room temperature varied between 24 and 30°C

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TABLE 2. EFFECT OF WET AND DRY CONDITIONS, WITH AND WITHOUT OXYGEN PACKING ON THE SURVIVAL OF THE CLAM, *MERETRIX CASTA*, AT 15°C (PERCENTAGE SURVIVAL)

Storage period, days	Wet pack				Dry pack			
	With oxygen		Without oxygen		With oxygen		Without oxygen	
	At 15°	At RT*	At 15°	At RT*	At 15°	At RT*	At 15°	At RT*
1	100	100	100	96	100	96	100	100
2	100	100	100	92	100	80	100	96
3	100	96	100	88	100	40	100	40
4	100	92	100	20	100	32	100	0
5	100	0	100	0	100	0	100	-
6	100	-	100	-	96	-	96	-
7	96	-	92	-	96	-	80	-
8	92	-	92	-	0	-	80	-
9	0	-	0	-	0	-	0	-

*RT-Room temperature varied between 24 and 30°C

survival. This is in agreement with the observations made on the clams *Villorita* species by earlier workers.

A more practical means of storing and transporting live clams would be an insulated box. In such a box, the desired temperature range of 10-20°C was readily obtained by preventing ice from coming into direct contact with the animals. The ice-melt water could be collected straight away in the bag itself. But re-icing became necessary after a day in order to maintain the temperature. As summarized in Table 3, the survival period was once again significantly enhanced by reducing the temperature. This is probably due to the reduced metabolic activity of the clams at lower temperatures. As observed in the previous trial, addition of water or packing under oxygen had little influence on improving the survival period.

TABLE 3. EFFECT OF ICING IN INSULATED BOX IN WET AND DRY CONDITIONS, WITH AND WITHOUT OXYGEN PACKING ON THE SURVIVAL OF THE CLAM, MERETRIX CASTA

Storage period, days	Wet pack				Dry pack			
	With oxygen		Without oxygen		With oxygen		Without oxygen	
	In ice+	At RT*	In ice+	At RT*	In ice+	At RT*	In ice+	At RT*
1	100	100	100	100	100	100	100	100
2	100	100	100	100	100	100	100	100
3	100	100	100	96	100	92	100	84
4	100	100	100	48	100	84	100	40
5	100	60	100	0	100	0	100	0
6	100	0	100	-	100	-	100	-
7	ND	-	ND	-	ND	-	ND	-
8	80	-	88	0	84	0	76	-
9	0	-	0	-	0	-	0	-

*RT-Room temperature, varied between 24 and 30°C

+-Temperature varied between 11 and 22°C

ND-No Data

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RESEARCH NOTE

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Quality Changes in Banana (*Musa acuminata*) Wines on Adding Pectolase and Passion Fruit

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Effects of pectolase and sodium metabisulphite addition on quality attributes of banana (*Musa acuminata,* Lacatan cultivar) wines were investigated. Wine treatments were banana (B); banana and pectolase enzyme (BE); banana, pectolase enzyme and sodium metabisulphite (BEM) and banana, passion fruit, pectolase enzyme with sodium metabisulphite (BPEM). Inclusion of the enzyme improved the clarity of wines but lowered taste and aroma scores. Addition of sodium metabisulphite produced a more intense yellow wine. As fermentation progressed, alcohol content increased, total soluble solids decreased and wines were less bright. Banana wine, BPEM had the best clarity (99.8 % transmittance), highest colour score (like moderately to like very much), highest overall score (like slightly to like moderately) with an alcohol content of 13.65 % on the 4th week of fermentation. There were no differences ($p>0.05$), for most quality attributes of wines fermented longer than 3 weeks.

Keywords : Bananas, Passion fruit, Must, Fermentatin, Pectolase, Quality evaluation.

Bananas (*Musa* spp) represent one of the most commercially important fruit crops of the agriculture industry in the Caribbean (Monteverde 1996). According to Bosuner (1995), banana is a major export in 5 Caribbean countries viz Jamaica, Grenada, Dominica, St. Vincent and St. Lucia. However, tremendous quantities of bananas are lost either through over production and/or in sorting and grading to meet quality standards for the fresh fruit export market, which could otherwise be used for processing. Having to compete in a global market, banana producers are increasingly being faced with competition for the fresh fruits. Therefore, it is imperative that the Caribbean banana industries diversify their markets in processed products, thus giving added value to the fresh fruits. According to Korydylas (1990), the peel and pulp of ripe bananas contain appreciable amounts of fermentable sugars, which can be used for alcoholic fermentation. Ripe bananas contain approximately 75 % water with almost 80 % of the solids, consisting of mixtures of sucrose, glucose and fructose (Forsyth 1980). The present study was aimed at producing wines by utilising the surplus/and excess bananas and to investigate the effects of pectolase, sodium metabisulphite and length of fermentation on the quality attributes of the prepared wines.

Bananas : Musa acuminata, AAA group 'Cavendish' sub-group of 'Lacatan' cultivar were imported from St. Vincent, West Indies. Visible signs of changes seen in skin colour were considered in judging the ripeness of bananas and also changes in the ridges of the skin from angular to round (Ramana and Jayaraman 1994). The maturity stage of bananas was judged by their yellow colour flecked with brown (maturity stage 7, Koffi et al. 1990). Bramwell (1998) reported the colour of the peel of 'Lacatan' bananas as L - 72.5, a - 1.1, b - 42.9. Passion fruit (*Passiflora edulis* F. flavicarpa) concentrate (pH 2.7:36° Brix) was blended with the banana *puree* (treatment BPEM).

Fermentation : All fermentation treatments were carried out in duplicate and were as follows: bananas (B); bananas + pectolase enzyme (BE); bananas, pectolase enzyme and

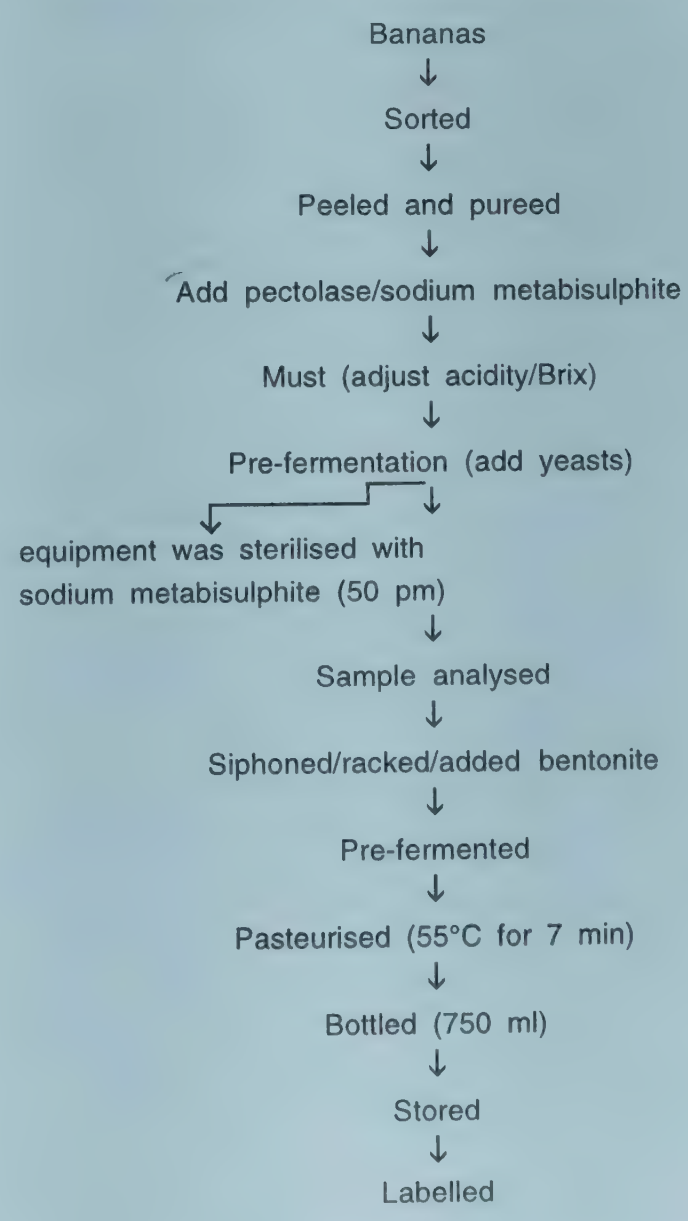

Fig. 1. Processing scheme for the production of banana wine

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sodium metabisulphite (BEM), and bananas, passion fruit, pectolase enzyme and sodium metabisulphite (BPEM). During primary fermentation at 7-day intervals, quality analyses (except sensory analyses) were performed to determine the shortest period necessary for the production of an acceptable wine.

Fig. 1. shows the processing scheme for the production of banana wines. Granulated sucrose was added to the peeled, pureed bananas and blended to obtain 23.0-23.5° Brix. Passion fruit concentrate was blended with the banana *puree* (treatment BPEM) and adjusted with sugar to obtain the desired °Brix. Commercial pectolase (0.15 %) containing pectolytic enzymes of pectin methyl esterase and polygalacturonase (WV14 8DL, Young's Home Brew Limited, Bilston, West Midlands) was added to must treatments BE, BEM and BPEM. Total titratable acidity (TAA) of the wine must was standardised with 0.60 % citric acid (# 22,060, AOAC 1990) to a pH of 3.3 ± 0.2. Sodium metabisulphite (120 ppm) was incorporated in some wine treatments (BEM; BPEM). Dried cultured wine yeasts (0.23%) *Saccharomyces cerevisiae* var ellipsoideus (# 67, Unicam Foods Limited, Norwich, England) and diammonium phosphate (0.2 %) were added to the must (21°C). Pre-fermentation was started in a 7 L food grade plastic wide-mouthed bucket with a loosely fitted lid for 7 days. The wine was aseptically siphoned into the sterile narrow-necked fermentation bottles (4.5 L), which were equipped with rubber lungs incorporating air locks, to which was added the bentonite clay (0.1 %) as a fining agent. The air locks were fitted with sodium metabisulphite (60 ppm) and refilled during the experiment. Primary fermentation was done at 20-21°C for 4 weeks. The wine was pasteurised at 55°C for 7 min and sodium metabisulphite (60 ppm) was added and filled into dark brown, long necked wine glass bottles (750 ml), with cork caps. Sensory evaluation was conducted on the 3rd and 4th weeks of fermentation.

Analysis : Wine colour (average of six readings) was measured on a Minolta Chromameter (Model CR-200B, Minolta Camera Corp. Ltd.). A 50 ml sample was transferred to a 100 ml colourless and transparent volumetric flask. The 8 mm measuring port of the Minolta Chromameter was placed at the bottom of the flask and the 'L', 'a' and 'b' co-ordinates were recorded. The 'L' value represented degree of lightness of colours (+/-a) degree of redness or greeness or (+/-b) the degree of yellowness or blueness. Total soluble solids (TSS) content as °Brix of the banana *puree*, must and wines was measured on a hand-held refractometer (Atago E. Type Series, Leica Inc.) and the average of three readings was recorded as °Brix. Total titratable acidity (TTA) of the wines was measured at weekly intervals during fermentation, using the method # 962.12, AOAC (1990). Clarity (average of two readings) of the wine was measured as percent light transmittance and was read at 470 nm on a spectrophotometer (Spectrome 20, Milton Roy Company) using distilled water as the standard with 100% transmittance. Alcohol content was measured by the difference in specific gravity of the must and wine. The % alcohol in the wine was calculated as outlined by Crabtree (1979) and Amerine et al (1980). Using the pour plate method, total viable plate counts (cfu/ml) in triplicate were performed for each wine treatment, using Plate Count Agar according to the method of Swanson et al. (1992). Plates were incubated at 35°C for 48 h.

Sensory evaluation : Panelists (17 members) drawn from staff and students of the University of the West Indies, St. Augustine were chosen on the basis of their experience and familiarity to the attributes of wines. Sensory evaluation was conducted in individual booths with white fluorescent light at the Department of Food Production, University of the West Indies, St Augustine. Each panelist was served with coded treatment wines (20 ml) served in clear longstem colourless glasses (30 ml). The wine samples were chilled at 8°C for 1 h prior to serving. Panelists were instructed to sip and rinse after each evaluation and to judge the wine samples on a 7 point Hedonic score (1 = dislike very much, 4 = neither like nor dislike, 7 = like very much). For each session, four treatments (B; BE; BEM; BPEM) were served. Replicates were served at a different session.

Statistical analysis : Data were analysed using Minitab Statistical Software (Minitab Inc.) and subjected to one- way analysis of variance at 5% level of significance, to investigate the effects of fermentation time on the quality attributes of wines.

Total soluble solids (°Brix) : TSS of wines decreased and % alcohol increased (Tables 1 and 2) on fermentation. There was greater utilisation of TSS in wines with sodium metabisulphite (BEM; BPEM, Table 1). The TSS of the banana pulp was 22.0 ± 0.50 °Brix. Data indicated that the rate of fermentation was highest during the first two weeks of primary

TABLE 1. EFFECT OF FERMENTATION PERIOD ON TOTAL SOLUBLE SOLIDS (°BRIX) OF BANANA WINES

	Fermentation time, weeks				
Treatment	0	1	2	3	4
B	23.5^{c}	16.0^{ab}	11.0^{a}	10.0^{a}	9.0^{a}
BE	23.0^{ab}	19.5^{ab}	16.0^{a}	13.0^{a}	9.5^{a}
BEM	23.0^{b}	17.5^{b}	8.0^{a}	7.0^{a}	7.0^{a}
BPEM	23.5^{c}	14.0^{ab}	8.0^{a}	7.5^{a}	7.0^{a}

Any two means followed by the same letters in rows are not significantly different (P>0.05)

B- banana ; BE- banana + enzyme; BEM- banana + enzyme+sodium metabisulphite; BPEM- banana + passion fruit + sodium metabisulphite

TABLE 2. EFFECT OF FERMENTATION PERIOD ON ALCOHOL (%) OF BANANA WINES

	Fermentation time, weeks			
Treatment	1	2	3	4
B	7.13^{b}	11.82^{b}	13.71^{b}	13.82^{b}
BE	2.04^{a}	5.03^{b}	9.24^{b}	10.67^{b}
BEM	4.21^{a}	13.17^{b}	13.46^{b}	13.92^{b}
BPEM	6.95^{a}	13.37^{b}	13.66^{b}	13.65^{b}

Any two means followed by the same letters in rows are not significantly different (P>0.05)

B- banana ; BE- banana + enzyme; BEM- banana + enzyme+sodium metabisulphite. BPEM- banana + passion fruit + sodium metabisulphite

fermentation. Total dry matter and total soluble solids have been important parameters in judging the ripeness of fruits (Skorikova et al. 1992) and in this experiment, the control TSS content of the banana must (23.0-23.5°Brix) was lowered as the yeast utilised the fermentation sugars, with the production of alcohol.

Total titratable acidity : In all the wines, TAA decreased significantly ($P<0.05$) on fermentation from 0.60 ± 0.01 % on week 0 to 0.12 ± 0.20 % on week 4. There were no changes ($P<0.05$) in TAA (expressed as % citric acid) for each treatment between the 3rd and 4th weeks of fermentation. According to Ough and Amerine (1988), wines contain the acids of musts and a number of acids are produced during and after alchol fermentation. According to Kordylas (1990), in the early stage of fermentation, acid inhibits the development of spoilage bacteria and encourages the growth and acitivity of wine yeast. Also, acid influences the formation of flavour in wines, as too much acidity can cause tartnes and too little, leaves it stale and insipid.

Colour : 'L' values decreased on fermentation, indicating that the wines became less bright (Table 3). The higher 'L' values for treatment wines BEM and BPEM, as compared to treatments B and BE, indicated the significance of the antioxidant, sodium metabisulphite on colour. Banana wine BPEM, had the most positive 'b' value, indicating that the addition of the passion fruit concentrate containing β-carotene would have enhanced the yellow colour. Also, wine BPEM was given the highest sensory colour score of 6.43 i.e. like moderately to like very much. A polyphenoloxidase enzyme has been isolated from banana pulp, which can catalyse the oxidation of a variety of diphenolic substances. Dopamine was reported to be the most reactive substrate for browning in the banana pulp (Palmer 1971). The addition of sodium metabisulphite can control both enzymatic and non-enzymatic browning (Lambrecht 1994 ; Vamos-Vigyazo 1994).

Clarity : Generally, as fermentation progressed, wines became clearer as reflected by higher % transmittance. By week 4, the inclusion of enzyme, treatment BE had a clarity transmittance % value of 99.5 as compared to a value of 65 % for wine without the addition of enzyme (B). By week 3, treatment BPEM had the highest % clarity value (99.8) and was rated for clarity by panelists as liked slightly moderately (5.81). Thakur et al (1997) reported that there was less pectic content in passion fruit, *Passiflora edulis* S. (0.5 % pectic substances, wet weight) as compared to banana, *Musa acuminata* L. (0.7-1.2 % pectic substances, wet weight), which may have accounted for differences in clarity. Bramwell (1998) reported 0.30 % pectin as calcium pectate in the pulp of 'Lacatan' banana. The presence of pectin in wines can result in colloidal suspension, which can make filtration and juice extraction more difficult. Koffi et al (1991) showed that pectic enzymes can reduce viscosity and increase filtrability of banana *puree*. According to Rombouts and Pilnik (1978), pectic and celluloytic enzymes are commonly used in the industry to aid in juice clarification and increased yield. Also, the addition of the fining agent, bentonite aided in the settling of suspended banana particles and dried yeast cells, resulting in the improvement of clarity.

Sensory evaluation : There were no differences ($p<0.05$) in all sensory attributes for all wine treatments between 3rd and 4th weeks of fermentation. However, by week 4, treatments BPEM and BEM (with inclusion of sodium metabisulphite) were given higher sensory clarity scores of 6.13 and 6.10, respectively (liked moderately to liked very much), as compared to the scores for treatment B (5.63 - liked slightly to liked moderately) and treatment BE (4.80 - neither liked nor disliked to liked). Aslo, treatment BPEM was given the highest colour score (6.43 - liked moderately to liked slightly) by week 4 of fermentation, which was reflected by its Lab values, being more bright and yellow (Table 3) as compared to other treatments. Banana wine B was rated by panelists with the highest sensory taste score (4.67 - neither liked nor disliked to liked slightly) and aroma score (5.93 - liked slightly to liked moderately) on 4 weeks fermentation. Treatment BE was given the lowest taste score (2.53 - disliked moderately to disliked slightly), with aroma score (4.17 - neither nor disliked to liked slightly). Pilnik et al (1975) and McLellan et al (1985) reported of greater tissue breakdown, releasing more components, which could contribute to soluble solids in pectinase-treated juices from apples, pears, apricots and carrots, which may have contributed to lower taste and aroma scores assigned to wines with enzyme. Treatment BPEM had the highest overall score (5.50-liked slightly to liked moderately).

Microbiological evaluation : By the 2nd week of fermentation, the highest colony forming units per ml (cfu/ml) were observed in all treatments: 9.4×10^5 (B), $4.68 \times 106^{6+}$ (BE); 1.72×10^7 (BEM) and 4.42×10^6 cfu/ml, (BPEM), which decreased to less than 30 colonies in all treatments by the 4th week of fermentation. The inhibition of microorganism by sodium metabisulphite) at low pH and the high alcohol acted on microbial cell viability (Amerine et al. 1980).

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TABLE 3. CHANGES IN COLOUR BANANA WINES ON FERMENTATION

Fermentation time / Treatment	Week 1 Lab	Week 2 Lab	Week 3 Lab	Week 4 Lab
B	30.35^{ab}	27.15^{ab}	25.30^{ab}	24.40^{ab}
	0.65^{ab} ; 0.67^{ab}	0.35^{b} ; 0.13^{a}	-1.17^{ab} ; 3.95^{ab}	0.40^{b} ; 0.53^{ab}
BE	31.65^{ab}	25.85^{ab}	25.65^{ab}	24.65^{ab}
	-0.71^{ab} ; 1.17^{ab}	0.39^{b} ; -0.18^{a}	-1.30^{ab} ; 3.38^{ab}	0.30^{ab} ; 0.87^{ab}
BEM	31.85^{b}	23.75^{a}	24.65^{ab}	24.65^{ab}
	-0.83^{ab} ; 2.23^{ab}	0.58^{b} ; -0.08^{a}	-1.00^{ab} ; 4.11^{ab}	0.07^{b} ; 1.33^{ab}
BPEM	41.20^{c}	24.20^{a}	25.40^{ab}	25.0^{ab}
	-1.81^{ab} ; 9.70^{b}	-1.38^{b} ; 4.63^{b}	0.37^{b} ; 2.20^{ab}	0.10^{ab} ; 0.93^{ab}

Any two means followed by the same letters in rows are not significantly different ($P>0.05$)

B- banana ; BE- banana + enzyme; BEM- banana + enzyme+sodium metabisulphite; BPEM- banana + passion fruit + sodium metabisulphite

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RESEARCH NOTE

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Shelf Life and Microbilogical Quality of Selected Dairy Products

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The profile of organoleptic and microbiological qualities of four commercial dairy products namely, flavoured milk, buttermilk, butter and sweet *khoa* were studied during storage at 6-8°C. In terms of their organoleptic qualities, the product showed a decreasing trend during storage, which ranged between days and weeks depending upon the product profile. The microbial population in these products appeared to be on higher side and the same increased during storage. The study revealed a poor shelf life and deteriorating bacteriological quality of the product evaluated.

Keywords : Flavoured milk, Buttermilk, Sweet *khoa*, Butter, Organoleptic and microbial characteristics.

Milk availability considerably improves a storage market for processed dairy products such as baby foods, condensed milk, milk powder, malted milk food, ice cream and butter over the years. The production of these products is estimated to double or even treble within the next 4-5 years. Flavoured fresh milk drinks and fermented milks are popular all over the world. Many organised dairies in India have started producing and marketing artificially flavoured fresh milk products. There has been tremendous increase in their consumption in recent times due to their refreshing nature, good digestibility and nutritional quality (Jayaraman and Gupta 1989).

Consumers are continuing to look for foods, which provide convenience without sacrificing quality. (Sajilatha et al. 1994). Spoilage due to microorganisms started gaining importance not only in the context of export, but also for home consumption. Contamination due to microorganisms during processing, handling and packing should be prevented to improve the shelf life. The microorganisms can cause serious health hazards, when contaminated products are consumed (Sharma 1997).

Considering the availability of a variety of dairy products in the market, an attempt was made in the present study, to evaluate the keeping and microbiological qualities of a few selected products during storage at refrigeration temperature.

The samples selected in this study included :

(i) Five numbers each of freshly prepared commercial batch of flavoured milk and buttermilk and (ii) Six numbers each of freshly prepared commercial batches of butter and sweet *khoa*.

The samples were selected at random from a single lot from a retail shop and stored at 6-8°C for studies on their shelf life and microbilogical quality during storage. Depending upon the keeping quality, the dairy products viz., the flavoured milk and butter milk were tested for a period of five days and butter and sweet *khoa* were tested at a weekly intervals for a period of 6 weeks for organoleptic and microbiological quality parameters. The organoleptic qualities were evaluated by a selected panel of judges on a 10 - point Hedonic scale. Selected samples were enumerated for the visible counts of total bacteria and fungi according to the standard procedures (Kannan 1996).

Besides, the presence of coliform bacteria in the fresh samples of the products was probed according to the procedure given by Kannan (1996).

Organoleptic qualities of flavoured milk (Table 1) indicated that the appearance of flavoured milk was poor due to separation of the product into two layers. This may be because of poor homogenisation. The thick flavoured milk ultimately became thin, watery and curdled mass. The results indicated that the flavoured milk should be consumed on the same day of preparation.

The changes in organoleptic qualities of the buttermilk (Table 1) were drastic. The product became sour and got separated into curd and whey. The product was acceptable till the third day of storage.

The butter samples had a better quality, up to weeks (Table 1). From the third week, rancid flavour was highly pronounced. The high bacterial counts might be responsible for the development of off flavour. The flavour of butter was so delicate that the relatively small amount of bacterial growth may cause appreciable damage to the flavour.

In *khoa,* the changes in organoleptic characteristics were very slow (Table 1). The sweet *khoa* was acceptable, the sixth week of storage. This might be because the *khoa* is prepared by concentrating the milk and sugar added in high concentration, which acted as preservative and prevented the rapid growth of microorganisms.

The high bacterial population (Table 2) in the flavoured milk may be due to the poor heat treatment of milk and unhygienic handling of the product during preparation and packing.

In buttermilk, the increase in bacterial population was very rapid (Table 2). The fungal as well as yeast population were high even on the first day and increased gradually. The yeast counts were higher than fungal but lower than bacterial population. The bacterial and fungal population of butter samples were comparatively higher than sweet *khoa* samples (Table 2).

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TABLE 1 ORGANOLEPTIC QUALITIES OF SELECTED DAIRY PRODUCTS DURING STORAGE AT 6-8°C

Storage period, days	Products									
	Flavoured milk					Buttermilk				
	Colour and appearance	Flavour	Texture	Taste	Overall acceptability	Colour and appearance	Flavour	Texture	Taste	Overall acceptability
1	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0
2	4.5	7.5	8.7	5.3	8.7	7.5	7.7	7.5	8.7	6.7
3	2.0	4.5	4.7	2.3	6.8	6.0	5.3	5.2	6.8	3.2
4	1.0	1.0	2.3	2.8	4.3	3.5	3.5	2.8	4.3	1.3
5	1.0	1.0	1.3	1.4	1.5	1.3	1.3	1.5	1.5	1.0
	Butter					Sweet *khoa*				
1	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0
2	8.3	4.7	8.7	7.3	7.3	8.8	8.5	8.7	7.3	8.8
3	7.7	2.7	7.8	4.8	5.0	8.0	7.7	7.8	4.8	8.0
4	7.3	1.0	6.5	3.0	4.5	7.3	6.6	6.5	3.0	7.0
5	6.3	1.0	5.8	1.3	3.2	6.3	5.3	5.8	1.3	6.0
6	5.7	1.0	5.6	1.0	2.2	5.2	4.5	5.6	1.0	5.0

TABLE 2. MICROBIOLOGICAL QUALITY OF SELECTED DAIRY PRODUCTS DURING STORAGE AT 6-8°C

Storage period, days	Product			
	Flavoured milk		Buttermilk	
	Total bacteria	Total fungi	Total bacteria	Total fungi
	(Viable counts, cfu/g)			
1	6.5×10^4	1.4×10^2	8.0×10^4	4.0×10^2
2	1.3×10^5	2.7×10^2	8.5×10^4	5.0×10^2
3	4.6×10^5	3.7×10^2	9.5×10^4	8.0×10^2
4	4.8×10^5	5.5×10^2	6.3×10^5	1.1×10^3
5	6.1×10^5	6.8×10^2	7.0×10^5	1.4×10^3
	Butter		Sweet *khoa*	
1	7.5×10^4	2.9×10^2	-	-
2	1.7×10^5	3.6×10^2	1.5×10^4	-
3	2.1×10^5	3.4×10^2	2.5×10^4	3.0×10^1
4	2.1×10^5	8.9×10^2	8.9×10^4	1.0×10^2
5	3.0×10^5	5.9×10^2	1.5×10^5	1.5×10^2
6	3.4×10^5	6.7×10^2	2.3×10^5	2.7×10^2

The observations indicated that all tested samples except butter confirmed the presence of coliform bacteria.

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RESEARCH NOTE

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Response of Some Indian Maize Samples for Aflatoxin Production by *Aspergillus flavus* Strains

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Laboratory response of five maize varieties commonly marketed in Bihar State, India to aflatoxin-producing and -non-producing strains of *Aspergillus flavus* was studied. Despite the small number of samples studied, it was possible to separate the maize genotypes into three groups according to their response, efficiently producing aflatoxins (good substrates), no aflatoxins producing (poor substrates) and ambiguous, depending on the strain used as the inoculum.

Keywords : Aflatoxin B_1, Atoxigenic and toxigenic *Aspergillus flavus,* Indian maize varieties.

Aspergillus flavus Link ex Fries and *A. parasiticus* Speare, are the two closely related fungi, occurring worldwide in the air and soil and causing pre-harvest aflatoxin contamination in maize, peanuts, cottonseed and tree nuts. Aflatoxin production is related to a number of complex factors, such as the presence of strains of aflatoxigenic fungi, substrate susceptibility and environmental factors, the most significant of which are temperature and moisture. These factors affect the behaviour of both, plants and microorganisms. The toxicities of certain species of *Aspergillus* have been recognized strain-related traits, a fact, which allows for great variability in toxin-producing potential (Hesseltine et al. 1970). A review of the literature shows that maize genotypes exhibit differing levels of susceptibility to the attacks of storage fungi (Cantone et al. 1983). Zuber et al (1978) demonstrated low concentrations of aflatoxin B_1 in maize hybrids and high concentrations in crosses of the maize variety 'OH-545' with hybrids.

In India, Bilgrami et al (1982) observed differences in the responses of some maize varieties to both fungal growth and aflatoxin accumulation by *A. parasiticus.* These differences have been associated with qualitative changes in the characteristics of genotypes. The present study was aimed to evaluate the laboratory production of aflatoxins in five different maize varieties largely used by the farmers of Bihar State, India in the presence of toxigenic strains of *Aspergillus flavus.*

Fungal strains and growth conditions : Toxigenic ('AFAU 21') and non-toxigenic ('AFAU 30') strains of *A. flavus,* obtained from U.L. Diener, Alabama Agricultural Experiment Station, Auburn University, USA, were used in the present study. These strains were analysed in the laboratory on maize variety 'Ganga 2', using both intact and damaged kernels. Cultures were maintained in inclined tubes with potato-dextrose-agar (Difco Laboratories, Detroit, Michigan) and stored at 4°C. Conida were collected by flushing 5-day old cultures in a sterile 0.01 % solution of Triton X-100. Suspensions were adjusted to 4 x 10^3 spores/ml of 'AFAU 21' and 2 x 10^4 spores/ml of 'AFAU 30', as enumerated by a hemacytometer (Petroff Hausser Counting Chamber, The American Optical Co., Washington DC) and 0.5 ml was used for inoculation.

Inoculation of maize and incubation : Five types of maize varieties, which are used as best food supplement for poor people in general and domestic animals in particular were collected randomly in separate sterile polythene bags from various maize storage centres of Bihar State, India. They were analysed by AOAC (1980) methods to ensure that they were free of aflatoxins. Fifty grams of kernels of each variety were sterilized in 500 ml Erlenmeyer flasks at 15 pounds pressure for 15 min. Each sample was inoculated with the corresponding 0.5 ml spore suspension, at concentrations of 2000 spores of 'AFAU 21' and 10000 spores of the 'AFAU 30'. To provide sufficient moisture in the substrate, 4 ml and 8 ml of sterile distilled water were added to each flask after three and four days of inoculation, respectively. Five replications of each treatment of maize variety were performed. The flasks were incubated in the dark at 25°C for 7 days.

Extraction and quantification of aflatoxins : At the end of incubation period, aflatoxins were extracted and quantified by the method of Shotwell et al (1966). Maize samples were sterilized at 15 pounds pressure for 15 min, 500 ml of distilled water was added to each of the flasks and shaken for 5 min in a multiwrist shaker. Chloroform (500 ml) was then added to each flask and were again shaken for 30 min. The mixture was filtered through cotton in a funnel and the two layers formed were collected and the aqueous layer was again extracted with another 200 ml of chloroform. The chloroform layers were mixed together and 25 g of anhydrous sodium sulphate was added into it. The mixture was filtered through a Whatman No. 4 filter paper, the filtrate was evaporated to 10 ml and purified in a silica gel column according to AOAC (1980) method. The fraction obtained from the column was concentrated to 500 µl and was analysed by thin-layer chromatography by the method of AOAC (1980) for visual quantification. After the purification of extracts on preparatory plates, aflatoxin B_1 was quantified spectroscopically (Nabney and Nesbitt 1965).

Statistical analysis : Statistical analysis was performed using the criteria of Whitaker and Dickens (1979) for determining the total error associated with each reading. The

differences of the means were calculated by the Tukey method and the Student (LSD) method. In both cases, a 5 % level of significance was used (Whitaker and Dickens 1979).

The maize varieties studied showed various levels of efficiency of aflatoxin accumulation, when aflatoxin production was measured by spectrophotometry (Table 1). The analysis of their responses in the presence of highly toxigenic strain showed three varieties ('Lakshmi', 'Kanaka' and 'High star') as good substrates for aflatoxin production (>1255 µg/kg). The variety 'Kanchan 517' and the variety 'Suraj' were poor substrates for toxin production (< 670 µg/kg). When aflatoxin production was determined by visual comparison with a standard thin-layer chromatography (Table 2), the differences were less pronounced but still detectable. Nevertheless, statistical analysis revealed a significant difference in the response of maize genotypes to 'AFAU 21'.

TABALE 1. SPECTROPHOTOMETRY DETERMINATION OF AFLATOXIN B_1 IN DIFFERENT VARIETIES OF MAIZE TREATED WITH TOXIGENIC ('AFAU 21') AND NON-TOXIGENIC ('AFAU 30') STRAINS OF *ASPERGILLUS FLAVUS*

Genotype	Aflatoxin, µg/kg[a]			
	'AFAU 21'		'AFAU 30'	
	Mean	Standard deviation	Mean	Standard deviation
'Kanchan 517'	650	479	47	31
'Lakshmi'	3250	1226	39	57
'Suraj'	670	385	70	92
'High star'	1255	835	517	569
'Kanak'	2375	916	21	37

[a]Average of 5 repetitions

TABALE 2. QUANTITATIVE VARIATIONS OF AFLATOXIN B_1 IN DIFFERENT VARIETIES OF MAIZE IN PRESENCE OF TOXIGENIC ('AFAU 21') AND NON-TOXIGENIC ('AFAU 30') STRAINS OF *ASPERGILLUS FLAVUS*

Genotype	Aflatoxin, µg/kg[a]			
	'AFAU 21'		'AFAU 30'	
	Mean	Standard deviation	Mean	Standard deviation
'Kanchan 517'	1247	789	72	47
'Lakshmi'	2570	1280	55	39
'Suraj'	925	619	61	70
'High star'	1725	1076	97	102
'Kanak'	2861	1771	0	0

[a]Average of 5 repetitions

TABALE 3. INTERACTION BETWEEN FUNGAL STRAIN AND MAIZE GENOTYPE

Genotype	Aflatoxin, µg/kg[a]	
	'AFAU 21'	'AFAU 30'
'Kanchan 517'	29,49730[c**]	3,85590[b,c]
'Lakshmi'	49,40579[a]	5,07950[b]
'Suraj'	27,79339[c]	3,59920[b,c]
'High star'	35,07580[b]	3,90885[a]
'Kanak'	41,59640[a]	4,55876[c]

[a]Averages converted according to Whitaker and Dickens (1979)
** Numbers followed by the same letter not significantly different

When the different varieties of maize were inoculated with non-toxigenic strain of *A. flavus* ('AFAU 30'), it was observed that this fungus was capable of producing low levels of aflatoxin B_1 (Tables 1 and 2). The variety 'High star' continued to be a good substrate for aflatoxin B_1 accumulation with this strain, whereas 'Lakshmi', 'Suraj' and 'Kanchan 517' were poor substrates for toxin elaboration. The variety 'Kanak' proved to be the most toxin-resistant substrate. The statistical analysis of these results showed significant differences in the response of various maize varieties to the non-toxigenic *A. flavus* strain (Table 3).

The results of the present investigation showed that the ability of *A. flavus* strains to sustain aflatoxin production depended on the interaction between the characteristics of the maize and the fungal inocula. The maize variety 'High star' showed a uniform response of susceptibility to both strains of *A. flavus.* The 'Kanchan 517' and 'Suraja' varieties were equally stable in their response for resistance. The 'Kanak' and 'Lakshmi' varieties were susceptible only for the toxigenic strain of *A. flavus.* These responses indicated that some maize varieties had no inhibitors to aflatoxin production. The presence of such inhibitors as beta-ionone in maize has been suggested by various workers (Nagarajan 1972 ; Priyadarshini and Tulpule 1978 ; Wilson et al. 1981).

The majority of studies reported in literature concerning differences in the capability of maize genotypes to sustain aflatoxin production have been performed with *A. flavus* and *A. parasiticus* (Zuber et al. 1978 ; Wilson et al. 1981). However, it was found in the present investigation that when the non-toxigenic strain of *A. flavus* was present in high levels of inoculum with various varieties of maize, it produced low levels of aflatoxin B_1 in most samples. The fact that some genotypes vary in their resistant/susceptible response in the presence of this strain could lead to more rigorous selection. Two of the maize varieties maintained their resistant response through all repetitions and with both strains of *A. flavus.*

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RESEARCH NOTE

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Standardization of Test Procedure for the Identification of Cold Slaughtered Meat

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Experiments were conducted to develop a test, which differentiates cold slaughtered meat from live slaughtered meat. The test was based on the reaction between the haemoglobin with malachite green solution, as there was high blood content in the subcutaneous blood vessels due to the minimum or no bleeding in case of cold slaughtered meat. The test procedure was standardized by altering various parameters like the reagents, their concentration and size of the sample. A large number of samples (30) were checked to establish the utility/efficiency of the test and it was found to be satisfactory.

Keywords : Cold slaughtered meat, Live slaughtered meat, Haemoglobin, Malachite green.

Meat is highly nutritious due to high quality proteins and amino acids and is in great demand throughout the world. It is obtained by slaughtering the healthy live animals and known as live slaughtered meat (Lawrie 1966). Cold slaughtered meat is the meat obtained by slaughtering the dead animal. Due to malpractice in ante-mortem and post-mortem inspections, carcasses from the dead animals may also reach the main stream of meat retail outlet.

Generally cold slaughtered meat is considered to be unfit for consumption. It could pose health problem to the consumer depending on the cause of death of the animal. If it is due to infection or disease, the consumer will be at risk by consuming such meat containing harmful organisms, its toxins and also the drug residues/drug resistant strains (pathogenic as well as non-pathogenic) (Brown 1982).

If the death of the animal is due to stress/strain/excitement during transport, undesirable changes occur in the absence of the rigor mortis and also due to the incomplete bleeding and such meat is perishable, unlike the meat derived from the live slaughtered animal (Gracey 1986). Therefore, it is very much essential to identify the cold slaughtered meat in order to safeguard the consumer health and to prevent economic loss for the meat industries and institutional catering like Army, Restaurants etc.

The main objective of this investigation was to develop a simple rapid test to identify the cold slaughtered meat. A method has been standardized to carry out a qualitative test to differentiate cold slaughtered meat from live slaughtered meat and this test is based on the principle that oxidized haemoglobin forms a green coloured complex with acid malachite green (Thornton 1968; Anon 1977) as there is high blood content in the subcutaneous blood vessels due to the minimum or no bleeding in the case of cold slaughtered meat. Cold slaughtered meat and the live slaughtered meat samples were procured from the local market and a slaughter house.

Preparation of the reagent : Aqueous solution of malachite green reagent (1 %) was prepared and it was further diluted to get 0.1 and 0.01% solutions. For one set of solutions of all the concentrations (0.01 %, 0.1 % and 1 %), pH was adjusted to 2.9 and other 5.3.

Samples : Meat from live and cold slaughtered sheep was obtained from a slaughter house. Live and dead animals were also slaughtered in the laboratory for conducting the test. Samples from the various meat stalls were procured.

Sample preparation : One part of the meat sample was macerated with 2 parts (wt./vol.) of distilled water and the supernatant was used.

Test procedure : An aliquot of the sample extract was mixed with a few drops of hydrogen peroxide and different volumes (0.1, 0.2, 0.4 and 0.7 ml) of various dye concentrations (0.01 %, 0.1 % and 1 %) of both the sets i.e., one set of dye having pH 2.9 and the another set having pH 5.3 and the results obtained are shown in Table 1. It was found that within 10 min. after the addition of the sample, the blue colour of the reaction mixture was changed to green in cold slaughtered meat, whereas it remained unchanged in the case of live slaughtered meat.

Difference in colour between the reaction of live slaughtered and cold slaughtered meat could not be made out clearly, when 0.01 % and 1 % of the reagents were used, as the original colour of the reagent itself was too light and too dark because of its respective very low and high concentration of the dye. But the slight change in the colour from blue to green could be made out, when 0.4 ml of the reagent with 0.1 % of the dye was used, but the colour complex formed was unstable and this could be due to the powerful oxidizing effect of hydrogen peroxide. Hence, experiments were repeated using different volumes of the reagent with 0.1 % dye, with and without the addition of hydrogen peroxide. Results were encouraging and the green colour formed with cold slaughtered meat was distinctly differentiable and was stable for more that 24-48 h, even the blue colour formed from the live slaughtered meat was also stable for more than 24-48 h. No significant difference was found in the final result of the reaction with pHs 2.9 and 5.3.

To avoid the cumbersome procedure and to make the experiment more easier to handle in field conditions.

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experiments were carried out with meat as such instead of using the meat extract. Since 0.1 % of dye solution was giving more encouraging results, the experiments were carried out with different volumes of malachite green reagent. Meat samples of different sizes like 1g, 2 g and 5 g were used. The reaction mixture contained 10 ml of distilled water, meat sample, 0.2 ml and 0.4 ml of 0.1% reagent. The pH of dye solution was adjusted to 5.3. Experiments were carried out both with and without hydrogen peroxide and the procedure was followed (Fig. 1).

The results obtained are tabulated in Table 2. Out of the different sizes of samples tested, 1 g and 2 g gave similar results and one can clearly distinguish live slaughtered and cold slaughtered meat with 0.4 ml of 0.1 % dye solution (pH = 5.3). As mentioned earlier, cold slaughtered meat formed a green coloured complex and the blue colour of the reagent remained unchanged in case of the live slaughtered meat. But, the colour was fainter as well as unstable in both live slaughtered and cold slaughtered meat, when hydrogen peroxide was used. When 5 g of the meat was used, the

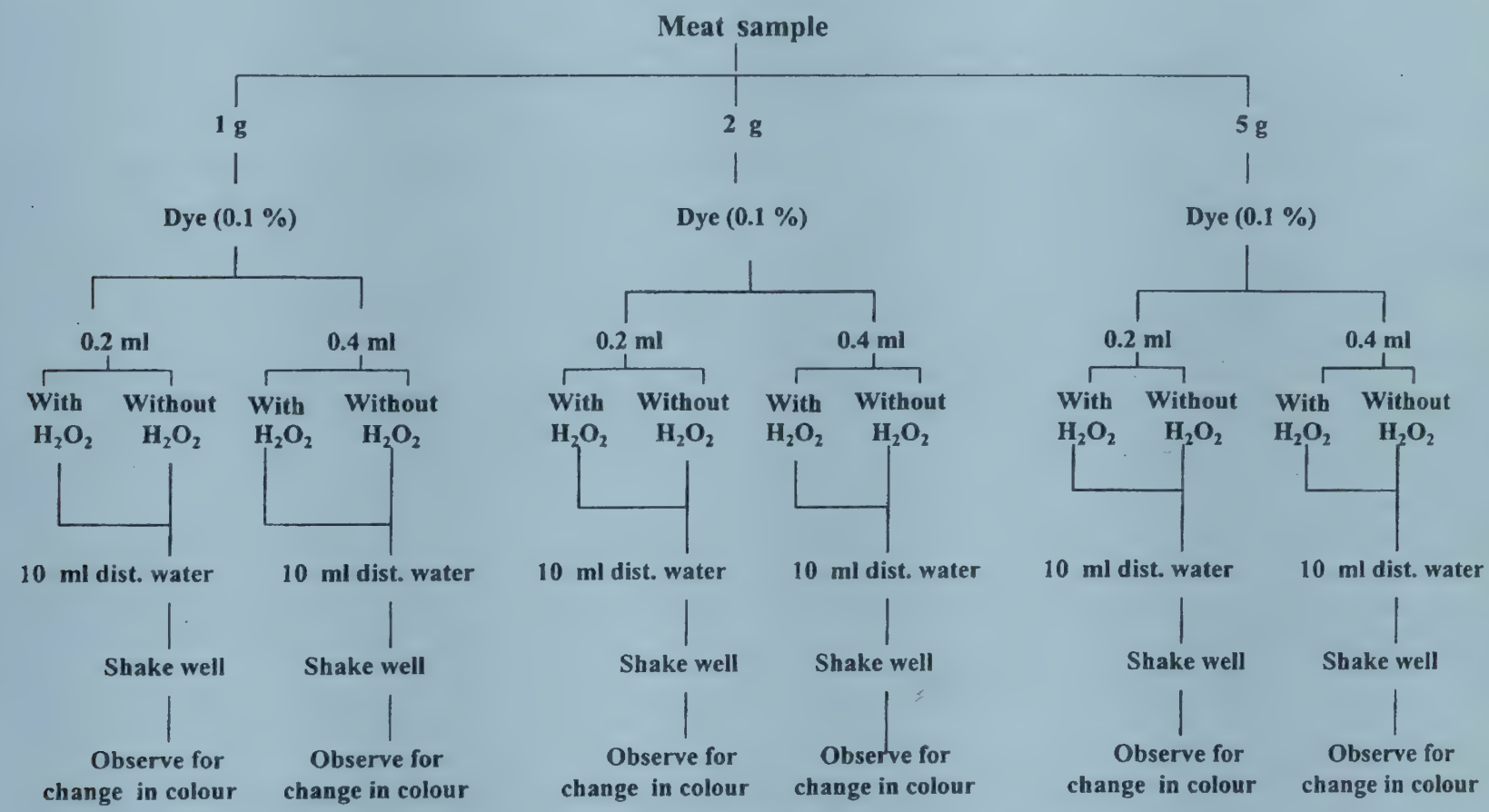

Fig. 1. Protocol for conducting the test with meat sample

TABLE 1. STANDARDIZATION OF TEST PROCEDURE WITH MEAT EXTRACT

Reagent concn.%	pH	Reagent vol ml	Reaction with H_2O_2 Colour reaction *LS	**CS	Colour stability, h LS	CS	Reaction without H_2O_2 Colour reaction LS	CS	Colour stability, h LS	CS
0.01	2.9	0.1	Pale blue	Colourless	Unstable	Unstable	Pale blue	Colourless	Stable for	Stable for
0.01	2.9	0.2	Pale blue	Colourless	after	after	Pale blue	Colourless	more than	morethan
0.01	2.9	0.4	Pale blue	Colourless	30 mins.	30 mins.	Pale blue	Colourless	18 hours	8 hours
0.01	5.3	0.1	Pale blue	Colourless	Unstable	Unstable	Pale blue	Colourless	Stable for	Stable for
0.01	5.3	0.2	Pale blue	Colourless	after	after	Pale blue	Colourless	more than	morethan
0.01	5.3	0.4	Pale blue	Colourless	30 mins.	30 mins.	Pale blue	Colourless	18 hours	8 hours
0.10	2.9	0.1	Pale blue	Colourless	Unstable	Unstable	Pale blue	Colourless	Stable for	Stable for
0.10	2.9	0.2	Blue	Colourless	after	after	Blue	Colourless	more than	morethan
0.10	2.9	0.4	Blue	Green	30 mins.	30 mins.	Blue	Green	2 days	18 hours
0.10	5.3	0.1	Pale blue	Colourless	Unstable	Unstable	Pale blue	Colourless	Stable for	Stable for
0.10	5.3	0.2	Blue	Colourless	after	after	Blue	Colourless	more than	morethan
0.10	5.3	0.4	Blue	Green	30 mins.	30 mins.	Blue	'Green	2 days	18 hours
0.10	2.9	0.1	Dark blue	Dark blue	No change	No change	Dark blue	Dark blue	Dark, no	Dark, no
0.10	2.9	0.2	Dark blue	Dark blue	No change	No change	Dark blue	Dark blue	change	change
0.10	2.9	0.4	Dark blue	Dark blue	No change	No change	Dark blue	Dark blue		
0.10	5.3	0.1	Dark blue	Dark blue	No change	No change	Dark blue	Dark blue	Dark, no	Dark, no
0.10	5.3	0.2	Dark blue	Dark blue	No change	No change	Dark blue	Dark blue	change	change
0.10	5.3	0.4	Dark blue	Dark blue	No change	No change	Dark blue	Dark blue		

[*LS = Live slaughtered; **CS = cold slaughtered]

TABLE 2. STANDARDIZATION OF TEST PROCEDURE WITH MEAT SAMPLE

(pH of dye solution = 5.3)

Sample size, g	Reagent concentration, %	Vol. of reagent, ml	Reaction with H_2O_2 Colour reaction *LS	Colour reaction **CS	Colour stability, h LS	Colour stability, h CS	Reaction without H_2O_2 Colour reaction LS	Colour reaction CS	Colour stability, h LS	Colour stability, h CS
1	0.1	0.2	Blue	Colourless	Unstable after 30 min.	Unstable after 30 min.	Blue	Colourless	Stable for more than 24 h	Stable for more than 8 h
	0.4	0.4	Blue	Green			Blue	Green		
2	0.1	0.2	Blue	Colourless	Unstable after 30 min.	Unstable after 30 min.	Blue	Colourless	Stable for more than 24 h	Stable for more than 8 h
	0.4	0.4	Blue	Green			Blue	Green		
5	0.1	0.2	Not readable	Not readable	Not readable	Not readable	Not readable	Not readable	Not readable	Not readable
		0.4								

[*LS = Live slaughtered; **CS = cold slaughtered]

coloured change could not be visualized, as the large volume of the reaction mixture was occupied by the sample.

Conclusion

A clear-cut and distinctly different reactions with live slaughtered and cold slaughtered meat were obtained, when 1 g to 2 g of meat sample as such was added to the reaction mixture, containing 0.4 ml of 0.1% dye solution with pH 5.3 without the addition of hydrogen peroxide.

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RESEARCH NOTE

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Comparison of Functional Properties of Hybrids and Varieties of Pearl Millet (*Pennisetum americanum*)

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Several functional properties of flours of newly developed four hybrids and three varieties of pearl millets grown under similar agronomic conditions were examined. There were no large and significant ($P<0.05$) differences in the water and oil absorption values among the hybrids and varieties. Mean flour solubility values were significantly ($P<0.05$) higher in hybrids than in varieties, whereas small variations between these two groups of genotypes were noticed for the swelling power of flour samples. Significant differences ($P<0.05$) in the nitrogen solubility index (NSI) values were obtained among hybrids and varieties, although the magnitude of difference was small. The higher concentration of the flour was required to form a gel in case of varieties as compared with hybrids.

Keywords : Functional properties, Hybrids, Varieties, Pearl millet.

As a coarse grain cereal for human food, pearl millet sustains the lives of many millions of people, particularly of low income groups in several African and South Asian countries. It is considered a rich source of energy and minerals in the daily diets of the people (Kumar and Chauhan 1993). Nutrient composition of pearl millet has been the subject of some studies in the past (Malleshi 1992, Malleshi and (Klopfenstein 1988).

To enhance utilization of pearl millet as composite flour in various food formulations, it is highly desirable to study and understand its functional properties. The functional properties are provided not only by the proteins of flours, but also by complex carbohydrate components of grains such as pectins and hemicelluloses (Martinez 1979). Relatively, less information is available on the functional properties of cereals, particularly of coarse grain cereals. Singh and Singh (1991) examined the functional properties of sorghum-peanut composite flour and suggested the implications of results in designing protein-enriched products based on sorghum flour, especially for sorghum growing regions of the world. The swelling capacity of flour influenced the *roti* quality of pearl millet (Subramanian et al. 1986). The functional properties of pearl millet are considerably influenced by the starch component, which is the principal constituent of the seed (Beleia et al. 1980). The chemical and functional properties of a limited number of millet flours were recently examined by Akubor and Obiegbuna (1999). In view of the fact that pearl millet is grown in a wide range of harsh environments, varieties and hybrids suitable for different environments are being continuously developed by the various breeding programmes in India and abroad. The present investigation was undertaken to compare the functional properties of newly developed hybrids and varieties in order to find out the differences among genotypes.

The newly developed and released hybrids and varieties of pearl millet, were obtained as a bulk seed lot from the Department of Plant Breeding, CCS Haryana Agricultural University, Hisar. Four hybrids 'HHB-60', 'HHB-67', 'HHB-94' and 'HHB-117' and three varieties 'HP-8601', 'MP-293' and 'HC-4' were used in the present study. These were grown during the 1998-99 rainy season under similar agronomic conditions at the experimental farm of the above mentioned department. All the grain samples were cleaned by removing all foreign materials. The cleaned samples were stored in a refrigerator at 5°C, until used. Whole grain samples were ground to a fine powder, when required for analysis and flour samples were used..

Functional properties

Water and oil absorption : The methods described by del Rosario and Flores (1981) were used for the determination of water and oil absorption with minor modifications. Sample (1 g) was mixed with 10 ml of distilled water or 15 ml oil for 30 min, the contents were allowed to stand for 30 min at 30°C in a water bath and then centrifuged at 3000 rpm for 20 min and the volume of the supernatant recorded to calculate the amount of water or oil absored by the sample. Refined groundnut oil was used for determination of oil absorption and results were expressed as g/g sample.

Gelation capacity : The gelation capacity was determined according to the method of Iyer and Singh (1997). Sample suspensions containing 8 to 15 % (w/v) flour in 0.5 % increments were prepared in 15 ml of distilled water. The test tubes were heated for 1 h in boiling water, rapidly cooled under running cold tap water and refrigerated for 3 h at 5°C. The least gelation concentration was determined as that concentration at which the sample did not fall down or slip from an inverted test tube.

Solubility and swelling power : For solubility measurement, 250 mg of flour was added to 10 ml of distilled water in a centrifuge tube. The contents were thoroughly mixed using a Vortex mixture and heated at 65°C for 30 min with occasional strirring, then centrifuged at 5000 rpm for 10 min. The supernatant (5 ml) was dried in a petri dish and soluble solids content was calculated as the percentage of flour soluble in water. The swelling power was determined by weighing the

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residue and calculating the increase in weight of the residue as the swelling power of the sample.

Nitrogen solubility index (NSI) : The nitrogen solubility index was determined at pH 6.8 according to the AOAC (1984) method. It was calculated as the ratio of the nitrogen soluble at pH 6.8 in a sample to that of the total nitrogen in the sample. Soluble nitrogen and total nitrogen in the sample were determined by the Kjeldahl method (AOAC 1984).

Gelatinization temperature : The gelatinization temperature of the sample (starch) was determined using a light microscope and a congo-red dye (0.2%) was used as a stain. The aqueous solution of flour sample was heated in a mini block heater and samples were taken from 65°C onwards at 1°C interval, until the gelatinization temperature was reached. The temperature at which 90% of the starch granules was stained, was recorded as the gelatinization temperature of the sample.

Statistical analysis : For statistical analysis, three replicates were used for the determination of each functional property. Standard error was determined by one-way analysis of variance (Snedecor and Cochran 1967).

The water absorption capacity of flours of pearl millet hybrids and varieties varied form 1.44 to 1.59´% and 1.42 to 1.59 %, respectively, indicating very small and non-significant differences among hybrids and varieties (Table 1). When expressed on protein basis, water absorption varied from 13.0 to 14.2% and from 11.8 to 13.5% for hybrids and varieties, respectively. Mean water absorption value on protein basis was significantly higher in hybrids than in varieties. Panwal and Pawar (1989) reported water absorption capacity of pearl milet flour and protein to be 1.85 and 13.50 g/g, respectively. The values for oil absorption of pearl millet flour were between 0.97 and 0.98 g/g sample for hybrids and between 0.97 and 0.99 g/g sample for varieties. This indicated no noticeable differences in the oil absorption capacity of hybrids and varieties of pearl millet. Significant differences ($P<0.05$) were observed in oil absorption of hybrids and varieties, when results were expressed on protein basis. The oil absorption of pearl millet flour appeared to be lower than those reported for sorghum by Singh and Singh (1991) and higher than those reported for wheat (Iyer and Singh 1997). These difference might have been due to the differences in the grain characteristics of the cereals. There were no significant differences in the swelling power of hybrid and varieties of pearl millet (Table 1). The mean value for swelling power was 1.15 g/g sample for hybrids and 1.14 g/g sample for varieties, indicating a small variation between these two groups of genotypes of pearl millet. The swelling capacity of flour of pearl millet has been reported to be positively correlated with the water-soluble proteins and water soluble starches (Subramanian et al. 1986). The starch granules are held together by hydrogen-bonding forces in the form of crystalline bundles and these structures are hydrated and eventually swelling takes place, when an aqueous suspension of starch granules is heated (Singh et al. 1989). Additional information on swelling pattern of pearl millet flours by heating at different temperatures would be very helpful in this direction.

Flour solubility of pearl millet hybrids and varieties ranged from 7.4 to 8.3 % for hybrids and from 7.0 to 8.1 % for varieties (Table 1). Mean flour solubility value was significantly ($P<0.05$) higher in hybrids than in varieties. The flour solubility values observed in the present study are similar to those reported by Panwal and Pawar (1989) and remarkably lower than those reported by Elkhalifa and Singh (1996). Generally, swelling and solubility patterns of flour samples of cereals and legumes provide information on the nature of starch and protein molecules held together by the associating forces within the grain.

As shown in Table 1, nitrogen solubility index (NSI) was the highest (27.9%) for 'HHB-67' and the lowest (22.7%) for 'HP-8601', a commonly grown variety of pearl millet. Significant ($P<0.05$) differences in NSI values were obtained between hybrids and varieties, although the magnitude of differences was small. Mean NSI value was significantly higher in hybrid than in varieties. The present results indicated no relationship between the flour solubility and NSI values of hybrids and varieties. Generally, it is expected that flour solubility is greatly influenced by the soluble carbohydrates and water soluble

TABLE 1. WATER AND OIL ABSORPTION, SWELLING POWER, FLOUR SOLUBILITY, NITROGEN SOLUBILITY INDEX (NSI) OF PEARL MILLET HYBRIDS AND VARIETIES

Hybrids/varieties	Water absorption, g/g flour	Oil absorption, g/g flour	Swelling power, %	Flour solubility, %	NSI, %
Hybrids					
'HHB-117'	1.44 ± 0.03	0.97 ± 0.03	1.12 ± 0.02	7.46 ± 0.20	26.78 ± 0.43
'HHB-94'	1.51 ± 0.02	0.98 ± 0.04	1.18 ± 0.04	8.30 ± 0.20	26.91 ± 0.43
'HHB-67'	1.55 ± 0.03	0.98 ± 0.01	1.17 ± 0.03	7.90 ± 0.41	27.89 ± 0.19
'HHB-60'	1.59 ± 0.05	0.98 ± 0.04	1.14 ± 0.02	7.40 ± 0.45	24.71 ± 0.60
Varieties					
'HP-8601'	1.59 ± 0.04	0.99 ± 0.05	1.15 ± 0.05	8.06 ± 0.29	22.69 ± 0.40
'MP-293'	1.58 ± 0.04	0.99 ± 0.05	1.13 ± 0.06	8.06 ± 0.26	26.63 ± 0.28
'HC-4'	1.42 ± 0.09	0.97 ± 0.07	1.15 ± 0.03	6.96 ± 0.27	24.40 ± 0.75
'F' test	NS	NS	S	S	NS
CD at 5 %	-	-	0.78	1.18	-

NS = Non-significant at $P < 0.05$ level; S = Significant at $P < 0.05$ level

TABLE 2. COOKING TIME, GEL CONSISTENCY, GELATION CAPACITY, GELATINIZATION TEMPERATURE AND EMULSIFICATION CAPACITY OF PEARL MILLET HYBRIDS AND VARIETIES

Hybrids	Cooking time, min	Gel consistency, mm gel spread	Gelation capacity, % flour	Gelatinization temperature, °C	Emulsification capacity, g/g Flour	Emulsification capacity, g/g Proteins
Hybrids						
'HHB-117'	17.00 ± 1.15	51.50 ± 0.28	8.66 ± 0.30	68.00 ± 0.16	1.43 ± 0.04	12.97 ± 0.43
'HHB-94'	20.00 ± 1.32	52.33 ± 0.30	9.22 ± 0.30	70.00 ± 0.28	1.50 ± 0.05	12.81 ± 0.40
'HHB-67'	18.00 ± 0.57	55.14 ± 0.51	9.70 ± 0.19	69.00 ± 0.40	1.54 ± 0.05	14.05 ± 0.44
'HHB-60'	17.00 ± 1.01	52.93 ± 0.17	9.66 ± 0.21	70.00 ± 0.28	1.30 ± 0.05	11.58 ± 0.54
Varieties						
'HP-8601'	19.00 ± 2.01	55.50 ± 0.27	10.16 ± 0.16	69.00 ± 0.86	1.63 ± 0.06	12.18 ± 0.41
'MP-293'	19.00 ± 0.32	54.16 ± 0.16	9.83 ± 0.16	68.00 ± 1.50	1.40 ± 0.05	12.15 ± 0.53
'HC-4'	16.00 ± 2.10	53.66 ± 0.16	9.83 ± 0.17	69.00 ± 0.87	1.38 ± 0.10	11.43 ± 0.93
'F' test	NS	S	S	NS	S	S
CD at 5 %	-	0.74	0.58	-	0.16	1.39

NS = Non-significant at P < 0.05 level; S = Significant at P < 0.05 level

proteins of grain samples. A highly positive correlation was noticed between the flour solubility values and water soluble protein fraction of pearl millet varieties (Subramanian et al.1986).

Pearl millet grits are boiled to a desirable softness before being consumed as *Khichadi.* The cooking time of whole grain sample of hybrids varied from 17 min to 20 min and of varieties from 16 min to 19 min, indicating that varieties may cook faster than hybrids (Table 2). Hydration capacity and hydration index were the most important parameters related with the cooking time of pearl milet varieties (Khairwal et al. 1997). However, in the present study, there was no noticeable relationship between the water absorption of the flour and the cooking time of whole grains of pearl millet hybrids and varieties.

The gelation capacity and gel consistency values for hybrids and varieties showed variable results (Table 2). There were no clearcut differences in the gel consistency values, even though the mean value was higher for varieties than for hybrids. The higher concentration of the flour was required to form a gel in case of varieties in comparison with hybrids, as revealed by the gelation capacities of these genotypes (Table 2). The gelling abilities of cereal flours appear to be a function of the nature and type of protein and starch and interactions between them (Iyer and Singh 1997). The gel consistencies expressed as gel spread of hybrids and varieties were remarkably lower than wheat and higher than pulses, as reported by Iyer and Singh (1997). This would imply that supplementation of pearl millet flour with wheat flour may be required for various food formulations to get the desirable viscoamylographic properties.

There were no significant differences in the gelatinization temperature of pearl millet hybrids and varieties. The gel temperature varied between 68 and 70°C for hybrids and between 68 and 69°C for varieties, showing no noticeable differences between them. The gelatinization temperature of isolated starches of pearl millet has been reported to range between 68 and 70°C (Beleia et al. 1980). The emulsification capacities of pearl millet hybrids and varieties did not show large differences, even though the differences were significant (P<0.05). When expressed on protein basis, the emulsification capacity showed values between 11.6 and 14.1 g/g sample for hybrids and between 11.4 and 12.2 g/g sample for varieties. Higher emulsification values are attributed to the higher protein contents of grain legumes (Iyer and Singh 1997). In the present study, there was no relationship between the protein content and emulsification capacity.

However, there were considerable differences between hybrids and varieties of pearl millet with respect to the nitrogen solubility index, flour solubility, gelation capacity, cooking time and emulsification values. As these results are based on analysis of a limited number of genotypes of pearl millet, further work will be useful in this direction.

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RESEARCH NOTE

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Phenolic Antioxidants for the Control of Some Mycotoxigenic Fungi

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Phenolic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate (PG) are low molecular weight hindered phenols, containing 1-3 hydroxy groups that are commonly used to prevent autooxidation of lipids in a veriety of food products. The efficacy of these phenolic antioxidants in the control of eight mycotoxigenic fungal strains was investigated. BHA was found to be highly effective compared to BHT and PG, as it inhibited the vegetative growth of seven out of eight fungi tested. Similarly, it inhibited secalonic acid and satratoxin production by *Penicillium purpurogenum* and *Stachybotrys atra*, respectively at 100 µg/ml, while rest of the fungi were inhibited at 250 µg/ml concentration. However, *A. terreus* was an exception, as it produced terreic acid even at 500 µg/ml. BHT completely inhibited the growth of *S. atra* at 250 µg/ml concentration. On the other hand, *A. terreus* produced patulin in the presence of BHT even at 1000 µg/ml. Propyl gallate was comparatively a mild inhibitor of mould growth and mycotoxin production.

Keywords : Antioxidants, Mycotoxins, Mould growth, Butylated hydroxyanisole, Butylated hydroxytoluene, Propyl gallate.

Consumers are increasingly interested in foods that are microbiologically safe. To achieve this, it is necessary to know more about the additives that have been used traditionally by the food industry. Mycotoxin producing moulds tend to spread aggressively on foodstuffs and can cause myctoxicosis at epidemic scale due to systemic and enormous varieties of toxic pharmacological activities of mycotoxins in animal systems (Berry 1988). Several methods have been suggested for the control of mycotoxin contamination (Draughtan and Churchville 1985). Phenolic antioxidants are commonly added to both human foods and animal feeds to protect unsaturated lipids and other materials from spoilage by oxidative degradation. (Raccach 1984). At the concentrations used in the food industry, they are generally regarded as safe (GRAS). Earlier, most work on the antimocrobial properties of BHA and BHT was focussed on bacteria and fungi such as *Aspergillus flavus* (Kabara 1981; Rohani 1999), but little is known about the antifungal activity of these compounds on other common mycotoxigenic fungi, such as other species of *Aspergillus* and species of *Penicillium*. In the present investigation, an attempt was made to assess the efficacy of antioxidants in checking the mould growth and mycotoxin production.

Toxigenic strains of *Aspergillus, Penicillium* and *Stachybotrys atra* used in the present study were isolated from spices and dry fruits (Giridhar and Reddy 1997, 1998). Stock cultures were maintained on Czapek's Dox agar slants. All the three phenolic antioxidants (BHA, BHT and PG) were obtained from s.d. Fine Chemicals Ltd., Mumbai, India. Preparation of spore suspensions, stock solutions of butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate (PG), their addition to respective 250 ml conical flasks (to make final concentrations of 100, 250, 500, 1000 µg/ml (drying these flasks in oven at 120°C, followed by cooling and addition of 50 ml liquid medium (rice flour medium for *Aspergillus flavus* Link, Capek's Dox medium for *S. atra* Cords, minimal medium for *A. terreus* Thom, SMKY medium for *A. ochraceus* Wilhelm, YES medium for *P. citrinum* Thom and *P. purpurogenum* Stoll and glucose-asparagine medium for *P. aurantiogriseum* Diereckx) into these flasks were carried out according to Fung et al (1977). Controls were maintained for all the fungi. The experiment was performed thrice. Flasks thus inoculated were incubated at 27-29°C. At the end of incubation period (15 days in case of *Penicillium* toxins, 10 days for aflatoxin B_1, 15 days for ochratoxin A, 25 days for satratoxin, 15 days for terreic acid and patulin), the mycelium from each flask was collected by filtration through pre-weighed Whatman No. 4 filter paper and dried to a constant weight at 120°C for 15 h. The weight of mycelium was then obtained by re-weighing the filter paper with dried mycelium. The aflatoxin B_1 (AOAC 1984) and other mycotoxins produced by different fungi were extracted and estimated by standard methods (Hou et al. 1970 ; Scott et al. 1970 ; Damodaran et al. 1973 ; Subramaniyan et al. 1978 ; Subramaniyan 1982 ; Rao et al. 1985). The standard mycotoxins for this study were obtained from Sigma, USA.

All the phenolic antixoidants (BHA, BHT and PG) were found to be effective from moderate to good in checking mycotoxin production (Table 1). BHA was highly effective, as it inhibited the vegetative growth of seven out eight fungal strains tested. *Penicillium purpurogenum* and *Stachybotrys atra* failed to grow in the presence of this compound. Aflatoxin B_1, patulin, ochratoxin A and penitrem B production by respective fungi were completely inhibited at 250 µg/ml of BHA. Similarly, *P. citrinum* required more than 250 µg/ml for checking citrinin production. Fung et al (1977) opined that BHA has a different effect on the toxigenesis of different fungal strains. The toxin producing ability was affected more by BHA for some strains than others. In the present study, the same pattern was also observed in case of BHT and PG. Butylated hydroxytoluene completely inhibited the growth of *A. flavus, P. citrinum* and *P. purpurogenum* at 1000 µg/ml. Though the vegetative growth of *A.ochraceus* and *P.aurantiogriseum* could be recorded at 1000 µg/ml concentration of BHT. *Stachybotrys atra* was sensitive towards this antioxidant and was totally inhibited Propyl gallate was comparatively a mild inhibitor of mould growth and toxin production. *S. atra* was totally inhibited at 100 µg/ml concentration. Though *A. ochraceus* could grow at

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TABLE 1. EFFECT OF PHENOLIC ANTIOXIDANTS ON GROWTH AND MYCOTOXIN PRODUCTION BY SOME FUNGI

Antioxidant Conc. (µg/ml)	fungus #Toxin	*A. flavus* Aflatoxin B_1**	*A. ochraceus* Ochratoxin A	*A. terrus* *Terreic acid	*A. terrus* *Patulin	*P.citrinum* Citrinin	*P. aurantiogri-seum* Penitrem A	*P.purpuroge-num* Secalonic acid D	*S. atra* Satratoxin
Control	DW	13.7 ± 0.216	12.5 ± 0.245	12.90 ± 0.653	13.6 ± 0.277	13.8 ± 0.522	12.2 ± 0.163	12.4 ± 0.653	9.9 ± 0.653
	Toxin	15.4 ± 0.081	+ 2	10.20 ± 0.899	11.5 ± 0.367	38.0 ± 0.106	10.4 ± 0.326	+	16.1 ± 8.57
BHT 100	DW	13.6 ± 0.245	9.24 ± 0.163	ND	13.1 ± 0.989	12.7 ± 1.3	11.9 ± 0.734	10.9 ± -.734	2.5 ± 0.571
	Toxin	14.5 ± 0.355	+ 1	ND	10.7 ± 0.890	16.0 ± 1.63	10.0 ± 0.368	+	15.0 ± 0.489
250	DW	11.2 ± 0.171	8.96 ± 0.735	ND	12.9 ± 1.04	11.9 ± 1.21	9.9 ± 0.734	10.4 ± 0.734	ND
	Toxin	12.9 ± 0.571	+ 1	ND	10.6 ± 0.489	15.0 ± 0.816	9.5 ± 0.244	+	ND
500	DW	10.0 ± 0.816	6.44 ± 0.359	ND	12.0 ± 1.06	9.4 ± 0.359	9.3 ± 0.571	9.5 ± 0.489	ND
	Toxin	4.2 ± 0.326	ND	ND	9.3 ± 0.571	13.0 ± 1.22	8.5 ± 0.326	ND	ND
1000	DW	ND	6.04 ± 0.032	ND	8.2 ± 0.489	ND	4.4 ± 0.163	ND	ND
	Toxin	ND	ND	ND	5.6 ± 0.837	ND	ND	ND	ND
BHA 100	DW	7.0 ± 0.163	2.86 ± 0.068	12.8 ± 1.776	13.0 ± 0.816	12.9 ± 0.317	11.5 ± 0.489	ND	ND
	Toxin	3.0 ± 0.16	+ 1	10.0 ± 1.224	10.0 ± 0.163	16.0 ± 0.734	9.1 ± 0.48	ND	ND
250	DW	ND	ND	12.82 ± 0.706	ND	6.68 ± 0.898	ND	ND	ND
	Toxin	ND	ND	9.50 ± 0.408	ND	8.00 ± 0.734	ND	ND	ND
500	DW	ND	ND	12.18 ± 0.550	ND	ND	ND	ND	ND
	Toxin	ND	ND	8.25 ± 0.117	ND	ND	ND	ND	ND
1000	DW	ND	ND	ND	ND	ND	ND	ND	ND
	Toxin	ND	ND	ND	ND	ND	ND	ND	ND
PG 100	DW	13.5 ± 0.408	12.16 ± 0.081	12.7 ± 0.604	13.4 ± 0.659	9.68 ± 0.489	11.4 ± 0.408	11.40 ± 0.244	2.5 ± 0.489
	Toxin	15.0 ± 0.489	+ 1	10.2 ± 0.489	10.6 ± 0.816	12.50 ± 1.08	10.0 ± 0.816	+	3.9 ± 0.816
250	DW	9.8 ± 0.489	11.34 ± 0.08	12.6 ± 0.408	13.0 ± 1.224	9.22 ± 0.163	10.0 ± 0.408	10.92 ± 0.734	ND
	Toxin	3.2 ± 0.571	+ 1	9.5 ± 0.489	10.0 ± 0.163	11.50 ± 0.571	9.5 ± 0.103	+	ND
500	DW	7.7 ± 0.571	9.06 ± 0.457	12.0 ± 0.190	12.1 ± 0.898	7.76 ± 0.489	9.5 ± 0.914	8.40 ± 0.653	ND
	Toxin	2.6 ± 0.138	+ 1	9.0 ± 0.816	8.9 ± 0.489	7.50 ± 0.571	9.0 ± 0.653	+	ND
1000	DW	ND	9.0 ± 0.653	ND	ND	ND	ND	ND	ND
	Toxin	ND	ND	ND	ND	ND	ND	ND	ND

Toxin µg/ml), *Toxin in ppb, ** Toxin in ppm, DW = dry weight(mg/ml), BHT = Butylated hydroxytoluene, BHA = Butylated hydroxyanisole. PG = Propyl gallate, ND = Toxin not detected

1000 µg/ml concentration, it failed to produce ochratoxin A. The toxicity of PG towards *A. terreus* and *A. ochraceus* was almost uniform in the range of 100 µg/ml, as the amounts of respective mycotoxin produced were not much different. The antifungal nature of BHA and BHT has also been reported earlier for species of *Aspergillus* (Thomson 1991) but on conidial germination of the fungus.

In the present study BHA was found to have more inhibitory effect not only on growth and toxin production of toxigenic strains but also inhibited sporulation to some extent (visual and microscopic observations) of toxigenic strains. The mechanism of inhibitory effect of phenolic antioxidants on growth and mycotoxin formation remains to be explained. Reduced oxygen tension in the medium created by the antioxidants could be one of the reasons for the inhibited growth and mycotoxin production by the aerobic moulds. Certain phenolic compounds have the ability to alter cell permeability. They also may interact with cell membrane protein to cause disruption of membrane structure. These activities probably accounted for the antimicrobial effects of phenolic antioxidants especially BHA (Fung et al. 1977). In general, the antimicrobial activity of phenolic antioxidants depends upon different factors such as microbial species/ strain, type and concentration of phenolic antioxidants, concentration of microbial challenge, combination of phenolic antioxidants, temperature, other food additives and food components (Raccach 1984).

In conclusion, BHA but no BHT and PG inhibited the growth and toxigenesis of most fungal strains. BHA should be further explored as an antifungal and antimicrobial agent in addition to its proven value as an antioxidant in foods. More detailed investigations are needed regarding the safety use of phenolic antioxidants in human food as protectants from mycotoxin contamination.

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RESEARCH NOTE

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Quality of Chicken Meat Patties Containing Skin and Giblets

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The effect of inclusion of skin and giblets in chicken meat patties at different levels on product quality was studied. Addition of skin and/or giblet resulted in increase in cooking loss, shrinkage in diameter, thickness and fat content of chicken patties. Presence of skin alone reduced protein contents of chicken patties. Ash content, pH and shear values were not affected. Incorporation of skin at 18-21 % and giblets at 12-14 % levels did not affect the quality attributes of chicken patties.

Keywords : Giblets, Skin, Patties, Chicken, Quality.

Increased egg production in India has also led to the greater production of spent poultry meat. The consumer preference to broiler meat is hampering the marketing of these spent birds. There is a greater need for the utilization of low value components like skin and giblets along with the spent hen meat in the poultry industry. Further processing of chicken meat into comminuted products such as patties has improved organoleptic preference (Kondiah et al. 1988; Nath et al. 1995). Patties made with chicken meat had higher colour and flavour scores than the duck meat patties (Prabhakara Reddy and Rao 1977).

The aim of the present study was to incorporate skin and giblets at different levels in chicken meat patties and evaluate their quality.

Twenty spent chicken aged over 18 months were slaughtered and dressed conventionally in four trials for this study. The carcasses were pressure-cooked (1 kg/cm^2) for 5 min. to facilitate easy debonning of meat. Four types of formulations were made by incorporating skin and giblets (heart, liver and gizzard) at different proportions as follows. Formulation F_1 with chicken meat alone 90 %. F_2 with chicken meat 69 % and skin 21 %, F_3 comprising chicken meat 76 % and giblets 14 % and the formulation F_4, with chicken meat 60 %, skin 18 % and giblets 12 %. Each formulation was added with vegetable oil 4 %, egg albumen 1.5 %, Maida flour 1.5 %, salt 2.0 % and spice mix 2 %. Meat and giblets were minced twice and for skin thrice by using a Hobard mechanical mincer with 8 MM plate. The minced meat was thoroughly mixed with the remaining additives. Patties of uniform circular shape of 7.98 cm diameter and 1.44 cm thickness each weighing 80 g were prepared by using a specially fabricated steel mould. The patties were cooked in an electric oven at 180°C for 30 min. Samples of each formulation were analysed for physical, physico-chemical, chemical and organoleptic qualities. Cooking loss, shrinkage in diameter, gain in thickness were calculated by measuring the values before and after cooking. Warner-Bratzle shear force, pH (Lakonen et al. 1970), waterholding capacity (WHC) (Wardlaw et al. 1973), Extract release volume (ERV) (Jay 1964), proximate composition (AOAC 1985), thiobarbituric acid (TBA) values (Witte et al. 1970), organoleptic evaluation on a 9-point Hedoxic scale were determined. The data collected in the above four trials was statistically analysed (Snedecor and Cochron 1968).

The patty formulations F_2, F_3 and F_4 had significantly ($P<0.01$) greater cooking loss and greater shrinkage in diameter compared to control (F_1) (Table 1). This may be due to more loss of water on cooking. Similar observations were noticed by Kondiah et al (1988) in chicken meat sausages with the inclusion of skin and giblets. Higher gain in thickness in F_2, F_3 and F_4 compared to control (F_1) may be attributed to more shrinkage in diameter in those formulations. Similar observations were made by Prabhakara Reddy and Rao (1975) in chicken meat patties. Addition of giblets and skin + giblets, however, had marginal ($P<0.05$) influence on the mean shear force values.

Formulations of F_3 recorded significantly ($P<0.01$) higher pH values and waterholding capacity (WHC) in both cooked and uncooked patties, whereas the F_2 recorded higher ($P<0.01$) ERV values. The least WHC in F_2 formulation may be due to its lower protein content, which in turn, might be due to the addition of skin. Presence of skin and higher fat content in F_2 samples could be responsible for higher ($P < 0.01$) ERV values. Cooked patties recorded ($P<0.01$) significantly higher pH and lower ERV and WHC values compared to the uncooked ones.

In the uncooked samples, the formulations, F_1 and F_3 had significantly ($P<0.01$) higher per cent moisture, while F_1, F_3 and F_4 recorded higher per cent crude proteins than F_2, whereas per cent ether extractives was ($P<0.01$) higher in F_2 formulation. Presence of skin alone in F_2 formulation was responsible for such increase of ether extractives and decrease of crude proteins. Ash content was not affected in the formulations. Cooked patties recorded significantly ($P<0.01$) lower percent moisture and higher crude protein, ether extractives and total ash contents. These findings are in accordance with those of Kondiah and Panda (1987) and Lyon et al (1988) in chicken patties.

Patties formulations containing skin and giblets gave significantly ($P<0.01$) superior juiceness (F_2, F_3, F_4) tenderness (F_3 and F_4) and overall acceptability (F_2 and F_3) scores than the others. Higher scores in the above parameters might be due to higher fat content in the above formulations, as also reported by Nath et al (1995) in chicken meat patties. In

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TABLE 1. EFFECT OF FORMULATIONS ON THE QUALITY CHARACTERISTICS OF CHICKEN PATTIES

Characteristic		Patti formulation			
		F_1	F_2	F_3	F_4
Cooking loss, %		$30.31^{a} \pm 0.27$	$33.43^{c} \pm 0.27$	$31.56^{ab} \pm 0.02$	32.18 ± 0.27
Shrinkage in diameter, %		$10.44^{a} \pm 0.14$	$12.54^{b} \pm 0.07$	$13.38^{c} \pm 0.04$	$13.09^{c} \pm 0.07$
Gain in thickness, %		$20.48^{a} \pm 0.38$	$22.83^{b} \pm 0.30$	$26.29^{d} \pm 0.07$	$24.21^{c} \pm 0.18$
Shear force values, kg/g		$0.24^{c} \pm 0.05$	$0.25^{c} \pm 0.05$	$0.22^{a} \pm 0.03$	$0.23^{ab} \pm 0.05$
pH	Uncooked	$6.08^{xb} \pm 0.02$	$6.10^{xb} \pm 0.02$	$6.14^{xa} \pm 0.02$	$6.17^{xb} \pm 0.02$
	Cooked	$6.14^{yb} \pm 0.01$	$6.17^{yb} \pm 0.01$	$6.20^{xa} \pm 0.01$	$6.23^{xa} \pm 0.02$
Water holding capacity	Uncooked	$49.16^{xb} \pm 0.23$	$39.07^{xd} \pm 0.31$	$54.57^{xa} \pm 0.35$	$44.15^{xc} \pm 0.32$
	Cooked	$46.32^{yb} \pm 0.21$	$35.40^{yd} \pm 0.36$	$49.16^{ya} \pm 0.31$	$40.82^{yx} \pm 0.38$
ERV values	Uncooked	$16.37^{xc} \pm 0.06$	$17.06^{xca} \pm 0.16$	$14.81^{xaA} \pm 0.10$	$6.31^{abA} \pm 0.25$
	Cooked	$13.81^{c} \pm 0.24$	$14.68^{CB} \pm 0.18$	$12.31^{ab} \pm 0.28$	$13.12^{abB} \pm 0.20$
Moisture, %	Uncooked	$58.15^{xb} \pm 0.23$	$53.43^{xa} \pm 0.30$	$59.33^{xb} \pm 0.20$	$54.59^{yab} \pm 0.30$
	Cooked	$44.96^{yb} \pm 0.28$	$42.70^{ya} \pm 0.41$	$44.35^{yb} \pm 0.27$	$43.41^{yab} \pm 0.17$
Crude proteins, %	Uncooked	$24.96^{xc} \pm 0.13$	$21.68^{xa} \pm 0.25$	$23.86^{xb} \pm 0.21$	$23.99^{xb} \pm 0.27$
	Cooked	$37.03^{yc} \pm 0.28$	$28.79^{ya} \pm 0.38$	$31.14^{yb} \pm 0.36$	$29.27^{ya} \pm 0.26$
Ether extractives, %	Uncooked	$13.18^{xa} \pm 0.08$	$19.23^{xc} \pm 0.17$	$14.58^{xa} \pm 0.13$	$17.60^{xb} \pm 0.16$
	Cooked	$17.84^{ya} \pm 0.34$	$25.83^{yc} \pm 0.30$	21.97 ± 0.30	$23.42^{yc} \pm 0.14$
Total ash, %	Uncooked	$2.39^{xa} \pm 0.05$	$2.16^{xa} \pm 0.21$	$2.41^{xa} \pm 0.11$	$2.34^{xa} \pm 0.04$
	Cooked	$3.46^{ya} \pm 0.03$	$3.28^{ya} \pm 0.04$	$3.46^{ya} \pm 0.15$	$3.15^{ya} \pm 0.07$
Colour scores		$5.97^{a} \pm 0.07$	$6.00^{a} \pm 0.06$	$5.72^{a} \pm 0.13$	$5.71^{a} \pm 0.14$
Flavour scores		$5.73^{a} \pm 0.22$	$5.85^{a} \pm 0.15$	$5.28^{b} \pm 0.32$	$5.26^{b} \pm 0.32$
Juiciness scores		$6.06^{a} \pm 0.14$	$6.26^{b} \pm 0.01$	$6.26^{b} \pm 0.10$	$6.25^{b} \pm 0.10$
Tenderness scores		$5.86^{a} \pm 0.09$	$5.60^{a} \pm 0.23$	$6.01^{b} \pm 0.12$	$6.00^{b} \pm 0.18$
Overall acceptability		$6.06^{a} \pm 0.05$	$6.26^{b} \pm 0.19$	$6.26^{b} \pm 0.05$	$6.00^{a} \pm 0.09$

Each value is a mean ± 5 D of 4 replicates

The mean values bearing the similar superscript in row (a,b,c,d) or in column wise (x,y) for each criterion do not differ significantly (P<0.01)

agreement with the findings of Thind et al (1988) in chicken patties, decreased colour (P<0.05) and flavour (P<0.05) scores were observed in F_3 and F_4 formulations, which might be due to the dark brown colour of giblets and hearts and the flavours released by them on cooking. However, the scores were within the levels of acceptability. The study indicated that skin and giblets can be incorporated in chicken meat patties up to the levels of 18-21 % and 12 - 14 %, respectively without affecting the sensory quality attributes.

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RESEARCH NOTE

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Effect of Peeling Methods on Breadnut (*Artocarpus altilis*) Seeds and Acceptability of Canned Breadnut Seeds in Brine

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Two methods viz., steam at atmospheric pressure and above atmospheric pressure (8 psi) for 5, 10, 15 and 20 min and use of hot (90 ± 2°C), 1 % and 10 % aqueous solutions of sodium hydroxide and 5 % and 10 % diammonium orthophosphate for 5, 10, 15, 20 and 25 min were employed to remove the peel from mature (80-90 days old) breadnut (*Artocarpus altilis*) seeds. Soaking in 5 % sodium hydroxide for 5 min, followed by a high-pressure water wash for 5 min resulted in the removal of the arils from the underlying membranes. Peeled seeds were canned in four different brine formulations to yield ready-to-eat products. Sensory evaluation revealed that breadnut seeds canned in 2.5 % brine, supplemented with sucrose (1.5 %) and calcium chloride (0.2 %) were most preferred ($P<0.001$) and yielded significantly ($P<0.001$), the most acceptable taste and texture than other formulations. Canned breadnut seeds were "commercially sterile", with a composition of 3.8 % proteins, 1.6 % fat, 24.8 % carbohydrates, 4.6 % minerals and 65.2 % moisture.

Keywords : Peeling, Thermal processing, Breadnut *(Artocarpus altilis)* seeds, Brine, Acceptability.

The breadnut is the seeded variety of the breadfruit and is also known by the name "Chataigne" in Trinidad, West Indies and pana de papita in Puerto Rico (De Bravo et al. 1983). The breadfruit tree is known by the scientific names, *Artocarpus communis, Artocarpus incisus* and *Artocarpus altilis*, with the last mentioned being the most widely accepted name (Barrau 1959; Stone 1974). Each seed contains a fairly rigid outer membrane (aril) and an inner fragile paper-like membrane, which envelops the fleshy white, edible portion of the seed (De Bravo et al. 1983). This genus is native to hot humid South-east Asia, where it is an important carbohydrate staple. Traditionally, in the West Indies, the mature peeled breadnut seeds are eaten after they are boiled in brine with the shell (aril) and underlying membrane or both the seeds and flesh are curried. Breadnut seeds constitute 30.1 to 46.8 % of the entire fruit (Quijano and Arango 1979; Tumaalii 1982; Dev Bravo et al. 1983; Wootton and Tumaalii 1984; Holloway et al. 1985). The crude protein content of the seed was found to be 13.3 %; the crude fat, 6.2 %; total carbohydrates, 76.2 %; and crude fibre, 2.5 %. Starch content of the seed was found to be 53.4 %, alcohol-soluble carbohydrates amounted to 16.4 %, and the ash content to 3.7 % (De Bravo et al. 1983). Compared to other tree nuts, the seed was found to contain high quantities of calcium, potassium, iron, niacin and riboflavin. Fruits of the breadfruit and breadnut are seasonal and highly perishable, with a shelf life of 2 to 3 days, after which they ripen and then deteriorate rapidly (Thompson et al. 1974; Maharaj and Sankat 1993). Processing can extend the availability of the breadnut seeds throughout the year and thus overcome the disadvantage of a short shelf life. Information on the preservation and processing of breadnut seeds is limited in the literature. Therefore, the present study was undertaken to select an effective peeling method and suitable brine formulation for canning of breadnut seeds to get an acceptable and ready-to-eat product.

Canning of breadnut seeds : Fully matured breadnut fruits (80-90 days old) were detached from backyard trees in North-East Trinidad, W I by using a 'cocoa knife' on a wooden pole. Seeds were manually removed from breadnut fruits and washed under running water (24-26°C). They were exposed to peeling treatments by steam at atmospheric pressure and above atmospheric pressure (8 psi) for 5, 10, 15 and 20 min, or chemically in hot (90 ± 2°C) aqueous solutions of 1, 5 and 10 % sodium hydroxide and 5 and 10 % diammonium orthophosphate for 5, 10, 15, 20 and 25 min. Control treatments were done by boiling the breadnut seeds in distilled water for 5, 10, 15 or 20 min. Effectiveness of the various peeling methods was judged by the ease with which the outer shell (aril) and inner underlying membrane were manually removed as compared to the control seeds. Degree of cooking was judged by the condition of the edible portion, as compared to the control sample. After peeling treatments, breadnut seeds were transferred to Mucilage Remover Device (Cocoa Research Unit, Trinidad, WI) to remove the loosened aril and the inner membrane by using a jet of water under pressure of 100 psi at varying rotation (7 rpm) durations of 3 min, 5 min and 7min. Peeled seeds (approximately 30 seeds) were rinsed in potable water (24-26°C), drained for 2 min and packed into 16 oz cans (453.6 g). Cans were filled with either 2.0, 2.5, 3.5 or 5 % sodium chloride solution. Cans filled with 2.5 and 3.5 % brine had 0.2 % calcium chloride. The head-space was set to 10 mm. Cans were steam-exhausted to an internal temperature of 85°C, sealed and steam-pressure retorted at 17± 2 psi for 20 min. cooled, and labelled. The average mass of sealed filled cans (12 cans) and drained weight of breadnut seeds and average vacuum of the cans were measured according to the methods of Ranganna (1977).

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Chemical analysis : Canned seeds in brine were drained for 2 min on a sieve and ground. Moisture, crude fat and ash (triplicate) contents were determined according to AOAC (1984) methods. Carbohydrate was calculated by difference. Titratable acidity, expressed as % citric acid and pH of the brine were measured instrumentally (Acumet No. 910, Fisher Scientific Instrument, Hampton, USA) according to the methods of Ranganna (1977).

Microbiological analysis : Microbiological tests were conducted according to the methods of Speck (1976). Breadnut samples were aseptically removed from cans and the presence/ absence of coliforms, using Violet Red Bile Agar medium (24 h/37°C; pH 7.4); lactic acid bacteria, using deMan, Rogosa and Sharpe medium (48 h/37°C; pH 6.2), *Staphylococcus aureus* in enriched medium with 2.5 % NaCl and 1.5 % sucrose (24 h/37°C; pH 7.2) and then streaked onto to Baird-Parker Medium enriched with egg yolk tellurite emulsion (24 h/37°C; pH 6.8); yeast and moulds, using acidified Potato Dextrose Agar (48 h/29°C; pH 3.5); and total aerobes on Plate Count Agar (48 h/32°C; pH 7.0).

Sensory analysis : Acceptability of the ready-to-eat breadnut seeds was determined by a 12-member semi-trained panel, chosen from staff and student members of the University of the West Indies. Panelists were served the samples in individual sensory evaluation booths located in the Food Quality Laboratory of the University of the West Indies and asked to indicate their acceptance for breadnut seeds on colour (acceptable or not acceptable); taste (too salty, acceptable or needed salt) and texture (too hard, acceptable or too soft) and to identify the preferred sample. For each sensory evaluation test, panelists were provided with four randomly labelled breadnut seeds on a white plate, one from each of the brine formulations (2.0 %, as formulation, there was 48 random order of presentation, presented to the 12 panelists, who were provided with a cup of water (24-26°C) for rinsing between tasting of each sample and a disposable plastic fork. The Chi-squared test was used for analysis of data (Lawless and Heymann 1998) to estimate whether the canned breadnut seeds were similar or different in various brine formulations, using a computer program NWA Statpak (Caribbean Agricultural Research and Development Institute 1994).

Peeling methods : Cracking of the aril was an indication of cooking, due to expansion of the enclosed edible portion as cooking progressed. Steam peeling either at atmospheric pressure or at 8 psi for 15 min resulted in the cracking of the aril, but there was difficulty in the removal of the aril from the underlying tissue. Steam peeling (8 psi) at atmospheric pressure for 15 min resulted in partial cooking of breadnut seeds within 6 mm from the surface of the seeds, while application of steam for 20 min resulted in overcooking and softening. Steam peeling at 8 psi for 5 min or 10 min resulted in the cracking of some seeds (12 % and 28 % respectively) and partial cooking (2 mm at 5 min; 5 mm at 10 min), but removal of aril from underlying tissue was difficult. Boiling of breadnut seeds (control) in water for 15 min resulted in the easy removal of aril and partial cooking up to 6 mm from the surface. Breadnut seeds were completely cooked by boiling for 20 min and were easy to peel manually.

Exposure to hot (90 ± 2°C), 5 % and 10 % aqueous solutions of diammonium orthosphosphate for 20 min resulted in partial cooking of the breadnut seeds with difficulty in cracking of arils and some discolouration of edible portion (5 % browning). Exposure for 25 min in both 5 and 10 % diammonium orthophosphate was effective in the separation of both membranes from the cells of the edible portion, resulted in complete cooking, and the edible portion of breadnut seeds was discoloured.

Chemical peeling, using hot (90 ± 2°C) 5 % sodium hydroxide for 5 min, followed by high pressure wash for 5 min gentle mechanical action in the Mucilage Remover Device, was the optimum and most suitable treatment for the removal of the aril and underlying membrane. The 3-min wash in the Mucilage Remover Device was adequate to remove the aril and inner membrane completely, but was insufficient for the removal of all traces of sodium hydroxide solution. This was manifested by the alkaline nature of the wastewater and by the soapy sensation felt on the surface of the seeds. The force of the water jet over the 7-min period, and the tumbling of the seeds in the rotating basket, broke some of the seeds (20 ± 3 %). Use of hot (91 ± 2°C) sodium hydroxide solution of 10 % at varying lengths of exposure resulted in easy removal of peel, but with varying levels of discolouration on edible breadnut seeds.

Chemical peeling takes advantages of the corrosive action of the chemical on plant tissues (Hanson 1976). The skins or peels are destroyed and as the solution diffuses inwards, edible portions may also get destroyed. Hence, the immersion time is critical to the efficiency of the process. The use of diammonium orthophosphate at 10 % and for 20 min as well as 5 and 10 % sodium hydroxide after 10 min and 15 min showed discolouration of the edible portion of the breadnut seeds. This discolouration may have been caused by oxidative browning of the cells of the edible portion or may have been due to the "dissolving" of the inner, brown, paper-like membrane. Although aril fragments were observed in these batches, there was no sign of the inner membrane.

Physical and chemical analysis : Average mass of sealed filled cans was 522.70 ± 1.8 g and average drain weight of breadnut seeds was 208.9 ± 2.6 g (approximately 30 seeds per can). The major nutrients of the cooked breadnut seeds were moisture 65.2 ± 0.98 %, fat 1.6 ± 0.27 %, protein 3.8 ± 0.08 % ash 4.6 ± 0.01% and carbohydrate 24.8 %. Crude fat 1.55 ± 0.27 % and protein content 3.76 ± 0.08 % were found to be less than that reported by De Bravo et al (1983) of 6.2 % and 13.3 %, respectively. The ash content of these breadnut seeds (4.60 ± 0.01 %) was found to be higher than that reported by De Bravo et al (1983) of 3.7 %. The pH of the brine was 5.05 ± 0.005 % with titratable acidity was 0.055 % citric acid.

Microbiological analysis : Canned breadnut seeds were microbiologically safe and free from potential spoilage organisms and pathogens. The results of the microbiological analysis also revealed that heat processing for 20 min at

TABLE 1. SENSORY RESPONSES TO COLOUR, TASTE AND TEXTURE OF BREADNUT SEEDS

Sensory Parameters	Colour		Taste		Texture	
Forumulation	Accept, %	Reject, %	Accept, %	Reject, %	Accept, %	Reject, %
1	81.25	18.75	56.25	43.75	50.00	50.00
2	85.42	14.50	89.58	10.42	89.58	10.42
3	89.58	10.42	79.17	20.83	60.42	39.58
4	81.25	18.75	33.33	66.67	60.41	39.59

Colour, not-significant; taste and texture are significant ($P<0.001$)

Formulation 1 brine containing 2 % NaCl only

Formulation 2 brine containing 2.5 % NaCl, 1.5 % sucrose and 0.2 % $CaCl_2$ only

Formulation 3 brine containing 3.5 % NaCl, 0.5 % sucrose only

Formulation 4 brine containing 5 % NaCl only

17 ± 1.4 in Hg was adequate to fully cook the seeds and render the can contents "commercially sterile", as nil growth was recorded on all petri plates. The average vacuum of the cans after 60 days (19.7 ± 0.6" Hg) was within the range of the average vacuum of cans taken one day after canning (19.6 ± 1.4" Hg). This suggested that there was no gas production due to microbial activity or due to a reaction of the brine ingredients with the material of the cans.

Sensory analysis : Table 1 indicates that formulation 2 (comprising 2.5 % sodium chloride, 1.5 % sucrose and 0.2 % calcium chloride) was the most ($P<0.001$) preferred formulation (66.7 % of the responses). There was no significant relationship ($P<0.05$) in acceptance for colour of breadnut seeds but significant differences ($P<0.001$) for taste and texture due to variations of brine formulation used in canning. Taste and texture were the most significant sensory parameters upon which preference was based. In terms of taste, formulations 2 and 3 were more acceptable (89.6 % and 79.2 % respectively) compared to formulations 1 and 4 (56.3 % and 33.3 % respectively). For formulation 2, only 2.1 % of the responses indicated that the breadnut seeds were too salty and 8.3 % responded that more salt was required. Formulation 2 had the most acceptable texture (43 responses out of 48 responses, 89.6 %), and was of moderate texture (neither too soft nor too hard), as only 4.2 % of the responses indicated that the breadnut seeds were too hard, while 6.3 % found that they were too soft. Formulations 3 an 4 were given the same scores for acceptability of texture (29 responses out of 48 responses), but a higher percentage of the responses (18.8 %).

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RESEARCH NOTE

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Sensory Evaluation of Different Levels of *Cadaba farinosa* (Dangarafa) in Kunun Zaki and Determination of its Position Among Other Saccharifying Agents

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The sensory characteristics of "Kunun zaki" prepared with different levels of *Cadaba farinosa* (Dangarafa) crude extract and its position among other saccharifying agents were determined. The concentration of the dangarafa crude extract at 0.25 % (v/v) level produced a more acceptable product with the highest mean scores in almost all the parameters studied. At a higher concentration (0.30 %), the extract imparted a pungent flavour with no pronounced effect on colour and total soluble solids. The mean scores for sweetness, flavour, mouthfeel and overall acceptability at the same concentration (0.30 %) however, decreased. The presence of *Cadaba farinosa* crude extract in "Kunun zaki" when compared with other saccharifying agents like malted rice, sweet potatoes, soybean seed and malted sorghum was found to have no significant difference ($p>0.05$) in colour and flavour. Further, the products from the different saccharifying agents were found to differ significantly at the same alpha level in sweetness, mouthfeel and overall acceptability.

Keywords : *Cadaba farinosa,* Kunun zaki, Saccharifying agents.

Kunun zaki is a cereal, based non-alcoholic beverage, prepared from millet (*Pennisetum typhoideum*); sorghum (*Sorghum bicolor or S. vulgare*) and occasionally maize (*Zea mays*) (Bolaji 1988). It is consumed by almost every Nigerian but has a wider acceptability in the Northern States. About 79% of the populace consume it on a daily basis and 21 % consume it now and then. Both in the urban and rural areas, it is marketed in appropriate packaging materials such as empty bottles of carbonated soft drinks, plastic kegs and bottles and in small polyethylene sachets. There are also depot centres, where this product is sold on a regular basis (Obekpa 1992).

The beverage is prepared locally with rudimentary equipment, with spices and other ingredients added. The product has a characteristic acid flavour and its nutritional qualities vary with batches (Adeyemi and Umar 1994). Principal among the other ingredients introduced during production are pastes of malted rice, malted sorghum and sweeet potato tubers (dry chips or fresh). Included in this group is a less prominent member, *Cadaba farinosa* popularly known as dangarafa (Hausa). Due to non-standaridization of production process, the ingredients are not added in specified quantities. All the ingredients are, however, known to aid in liquefaction and saccharification of the starchy slurry, making it more watery during Kunun zaki processing. The presence of starch hydrolysing enzymes in malted rice, malted sorghum and sweet potatoes has been documented (Kazuo et al. 1982; Andrew 1964 ; Douglas et al. 1982). The hydrolytic effect of *Cadaba farinosa* has been determined (Ayo 1998). *Cadaba farinosa* grows wild in Northern Nigeria and is found on termite mounds (Burkill 1975). The shrub is also cultivated in this region on a small scale and can live for as long as 20 years. In this study, the most acceptable level of the crude in Kunun zaki production and its position among other liquefying agents was determined.

Collection of plant material, grains and spice used : The dry stem of *Cadaba farinosa,* millet (*Pennisetum typhoideum*) grains and dry ginger (*Zingiber officinale*) were purchased from Yelwa-Tudu market in Bauchi State and used for the study.

Preparation of crude extract from Cadaba farinosa *:* The dry stem was cut into small pieces and 40 g were weighed. It was further chopped with knife and pounded using a mortar and pestle. This was mixed with distilled water (200 ml) and blended (Kenwood Industrial Blender, Model No. 33 BL 51, France) for 10 min. The crude homogenate was filtered using a muslin cloth and the filtrate was centrifuged at 10,000 **g** for 15 min. The supernatant was then used for the experiment.

Preparation of Kunun zaki samples : The millet grains and ginger (675 g and 7 g, respectively) were washed and soaked in tap water for 12 h. They were washed again at the end of the steeping period and ground into paste, using a local wet milling machine (abrasive milling). The slurry was divided into four equal portions into clean containers. To this was added boiling water (200 ml each) for gelatinization. The mixture was allowed to cool (65-70°C). To the gelatinised portions, varying concentrations (0.15; 0.20; 0.25 and 0.30 % v/v) of *Cadaba farinosa* crude extract were added separately and allowed to react for 12 h. The products were filtered into separate containers using a muslin cloth. Other Kunun zaki samples with millet as the main substrate were prepared using malted rice, malted sorghum, sweet potatoes and soybean seed as liquefying agents, following the traditional production process of Bolaji (1988). Each of the products was labelled appropriately and subjected to sensory evaluation with the most acceptable Kanun zaki with *Cadaba farinosa* extract above.

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TABLE 1. MEAN SCORES OF SENSORY EVALUATION OF DIFFERENT CONCENTRATIONS OF *CADABA FARINOSA* IN KUNUN ZAKI

Parameters	Crude extracts concentration, % v/v			
	0.15	**0.20**	**0.25**	**0.30**
Colour	7.63	7.66	7.74	7.67
Sweetness	6.04	6.30	7.04	6.48
Flavour	6.42	6.56	7.11	6.07
Mouthfeel	6.26	6.00	6.88	5.52
Overall acceptability	6.35	6.67	6.89	6.07
Total soluble solids	6.30	6.80	7.40	7.70

TABLE 2. MEAN SENSORY VALUES OF DIFFERENT INGREDIENTS USED IN KUNUN ZAKI

Parameters	Kunun zaki varieties				
	DSP	DMR	DDG	DSB	DMS
Colour	7.21	7.76	7.48	7.48	7.31
Sweetness	6.14	7.79	6.93	6.14	5.62
Flavour	6.21	7.00	6.34	6.07	6.00
Mouthfeel	6.03	7.48	6.72	5.97	5.76
Overall acceptability	6.48	7.69	6.62	6.45	6.24
Total soluble solids	6.00	7.50	7.50	6.00	6.03

NB. DMS - Kunun zaki with malted sorghum
DSB - Kunun zaki with soybean seed
DDG - Kunun zaki withCadaba farinosa
DMR - Kunun zaki with malted rice
DSP - Kunun zaki with sweet potato

Sensory evaluation : The products from varying concentrations of *Cadaba farinosa* were first subjected to consumer acceptability test, using a 9 point-Hedonic scale modified by Williams (1982) to choose the best product out of the four concentrations, involving 27 trained judges. The most acceptable level of concentration of the crude extract in the product was chosen and subjected to another sensory test with Kunun zaki products from the other four saccharifying agents. The judges were asked to evaluate the products for colour, sweetness, flavour, mouthfeel and overall acceptability. The total soluble solids of the 5 products were measured, using a refractometer (Sapelem 272.700). The mean scores were subjected to analysis of variance (ANOVA) at 0.05 % to detrmine the degree of difference between the samples (Gacula and Singh 1984).

Sensory evaluation of Kunun zaki *produced with various concentrations of* Cadaba farinosa *crude extract :* The mean scores for the sensory evaluation in each parameter are presented in Table 1. *Cadaba farinosa* crude extract at 0.25 % (v/v) produced the most acceptable Kunun zaki with the highest mean scores in all the parameters. The mean scores for colour remained fairly constant at all the concentrations (0.15-0.30 %). The scores for sweetness, flavour, mouthfeel and overall acceptability decreased above 0.25 %. The flavour imparted at higher concentration above 0.25 % was not liked by many. The mean values for the 5 Kunun zaki products using different liquefying agents are given in Table 2.

Statistical analysis on mean sensory values indicated that there were significant differences ($P<0.05$) between the samples in terms of overall acceptability, sweetness and mouthfeel. There was, however, no significant difference at the same alpha level ($P<0.05$) between the samples in terms of colour and flavour. The order of preference in overall acceptability was DMR, DDG, DSP, DSB and DMS, (Table 2).

The sensory evaluation of Kunun zaki produced with 0.25 % (v/v) of *Cadaba farinosa* crude extract had the highest mean scores in all the parameters evaluated except for colour, which remained higher at 0.3 % concentration. It would appear that the colour of the extract (light yellow) was masked by the dominant colour (grey) of the grains used as the raw material for the production. This agreed with the finding of Obekpa (1992), who reported the final colour of Kunun zaki samples to be mainly that of the raw material used. The pungent flavour imparted in the product by the crude extract above 0.25 % was the reason for rejection, although the total soluble solids (TSS) content at 0.3 % was still high.

The comparison in product acceptability using the 5 different ingredients showed that most people preferred Kunun zaki with malted rice. While the colour and flavour remained fairly constant in all the 5 different types, it appeared that the total soluble solids had a strong effect on sweetness, mouthfeel and overall acceptability. This could be explained by the fact that there existed inherent differences in the chemical nature of the ingredients used and the chemical imparted in the products might also be different. Moreover, the proportion of enzymes in each saccharifying agent and the total amount of reducing sugars produced in each case might be the likely factor, affecting the products and their overall acceptability.

It may be concluded that *Cadaba farinosa* can be used as a substitute in the production of Kunun zaki in place of other liquefying agents as it is cheaper and the pass a longer shelf life.

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RESEARCH NOTE

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Natural Contamination of Some Egyptian Fruit Juices and Beverages by Mycoflora and Mycotoxins

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The mycoflora analysis of some canned fruit juices and beverages showed a wide range of colony forming units (cfu) and species. Forty-four apecies and 2 varieties belonging to 15 genera were collected during the current study from mango, guava juices and grape, peach and apple beverages on Dicloran Rose-Bengal agar and 20 % sucrose-Czapek's agar at 28°C. The total counts of fungi widely fluctuated between 187-518 and 83-535 colonies/50 ml juice or beverages on the two isolation media. The highest contaminated product was guava juice and the lowest one was the natural grape beverages. The most prevalent fungi in all types of juices and beverages were members of *Aspergillus* and *Penicillium*. The thin layer chromatographic analysis of the chloroform extracts of the five different fruit juice and beverages (five each) revealed that all the five apple beverages tested were contaminated with aflatoxin B_1 and G_1 with concentrations ranging from 20-30 µg/L, while two out of the five guava juice samples tested were naturally contaminated with aflatoxin B_1 at 12 µg/L. All the samples of mango juice, grape and peach beverages were free from naturally occurring mycotoxins.

Keywords : Fruit juices, Fruit beverages, Mycoflora, Mycotoxins, Aflatoxins.

The soft fruits such as mango, guava, grape, apple and peach are highly susceptible to mould invasion and mycotoxin production in the field. Improper handling and storage conditions after harvest can also facilitate mould contamination. In processing the juices or beverages from fruits, some isolates of fungi have the ability to tolerate this processing. The high resistance of these organisms allowed them to produce their mycotoxins and spoil the beverages. Mycotoxins may enter the fruit juices or beverages by direct contamination, resulting from mould growth on the fruits, or by indirect contamination through the use of contaminated ingredients in processed fruits.

Now, there are several investigations focussed on the natural contamination of some fruits, vegetables and their products by mycoflora and mycotoxins (Rale and Vakil 1984; Wojtas-Koziel and Borecka 1990 ; Michailides 1991; Ugwuanyi and Jason 1991; Leistner 1992 ; Pokorn 1994; Beuchat 1995 ; Pao et al. 1996 ; Ejechi et al. 1998 ; Pao and Brown 1998). In Egypt, the information about the presence of mycoflora and mycotoxins in fruits and their products are very scarce (Moubasher et al. 1971; El-Kady et al. 1979). In this background, the present work was undertaken to determine the contamination level of mycoflora and mycotoxins in some Egyptian canned fruit juices and beverages.

Mycotoxin standards : All mycotoxin standards used throughout this study were kindly provided by Prof. Dr. I.A. El-Kady, Botany Department, Faculty of Sciences, Assiut University, Assiut, Egypt.

Collection of samples : Twenty-five samples of different fruit juices and beverages (5 each) were collected from shops and markets of different sanitation levels at Assiut Governorate, Egypt. These samples included canned juices of mango (*Mangifera indica*) and guava (*Psidium guajava*) and canned beverages of grape (*Vitis vinifera*), apple (*Pysus malus*) and peach (*Prunus persica*). The producing companies and ingredients of these samples are shown in Table 1. The samples were brought to the laboratory and kept in a refrigerator (3-5°C) till further analysis.

Isolation and identification of fungi : Requisite quantities of appropriate dilutions (in triplicates) of the samples were surface plated with Dicloran Rose Bengal medium-DRBC (King et al. 1979) and 20 % Sucrose-Czapek Dox agar (Moubasher 1993). The plates were incubated at 28°C for 1-2 weeks during which period the colonies of fungi were counted, identified and calculated per 10 ml for each sample. Fungal colonies showing characteristic morphology were isolated and subjected to identification (Raper and Funnell 1965 ; Moubasher 1993).

Mycotoxins analysis : Sample extracts for mycotoxin analysis were prepared from 50 ml quantities of each sample according to the method of El-Kady et al (1979). Chromatographic analysis of the chloroform extracts was achieved on percolated silica gel plate type 60 F 254 (Merck, Darmstadt) for the presence of aflatoxins B_1, B_2, G_1 and G_2, citrinin, diacetoxy-scirpenol, ochratoxins, patulin, rubratoxins, sterigmatocystin, T-2 toxin and zeralenone (Scott et al. 1970; Roberts and Patterson 1975 and Gimeno 1979).

Aflatoxins present in the extracts were quantitatively determined according to the method of Jones (1972). The aflatoxin concentration was estimated visually in the chloroform extracts by quantitative thin layer chromatogrophy by comparison with aflatoxins B_1, B_2, G_1 and G_2 standards. The detectable limit was about 5 µg/kg for aflatoxin B_1 and total aflatoxins (Przyhyeski 1975). For confirmation, the plates were sprayed with 50 % sulphuric acid in methanol (AOAC 1980).

Fungi recovered on DCRB : Thirty-five species and 2 varieties covering 13 genera were isolated from the five fruit products, when plated on DRBC. The total viable fungal propagules were found to range from 187-518 colonies/50 ml juice or beverages. The highest contaminated ssmple was guava juice and the lowest one was the natural grape

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TABLE 1 INGREDIENTS AND PRODUCING COMPANIES OF THE DIFFERENT-FRUITS AND BEVERAGES INVESTIGATED

Juice/beverages type	Ingredients	Producing company
Mango juice	Fresh fruits, natural mango, pulp, sucrose, water Total soluble solids = 18 %	Alexandria Confectionary and Chocolate Co., Hawamdieh Factories, Egypt
Guava juice	Guava pulp, sugar, citric acid Total soluble solids = 15 %	Edfina Co., Egypt
Natural beverages grape	Natural grape juice 20%, sugar syrup, natural colour, citric acid, natural aroma, vitamin C Total soluble solids = 12 %	Ismailia National Co. for food Industries (Foodico), Egypt, under licence from Wild-Heidelberg, Germany.
Natural beverages apple	Natural apple juice 20 %, natural colour, sugar, syrup, citric acid, natural aroma, vitamin C. Total soluble solids = 13 %	Ismailia National Co. for food Industries (Foodico), Egypt, under licence from Wild-Heidelberg, Germany
Peach beverages	Peach core, sugars, water, citric acid, sodium benzoate, synthetic colour flavours	Edfina Co., Egypt

beverages (Table 2). Rale and Vakil (1984) reported variable counts of fungal colonies in guava fruits, when plated on modified Czapek agar, malt extract-yeast extract glucose peptone agar and modified molybdate agar.

The distribution pattern of fungal species in fruit juices and beverages, when plated on DRBC is presented in Table 2. *Aspergillus* spp. was found to be the most predominant organism showing a 100 % frequency in almost all the samples analysed. Of the 11 *Aspergillus* spp. and 2 varieties isolated, *A. flavus, A. fumigatus, A fumigatus* var. *albus, A. niger* and *A. terreus* were the common species. A frequency of 79.5 % of *Aspergillus* was reported in a study, relating to parasitic and fungal contamination of fruits and vegetables (Peter et al. 1990). Ugwuanyi and Jason (1991) reported that about 17 % of mango fruit samples contained heat resistant fungi including *A. flavus.*

Penicillium spp. also exhibited a similar degree of frequency to that of *Aspergillus* spp. Among the 10 species isolated, *P. duclauxii* and *P. funiculosum* were more common in occurrence. Combrink et al (1985) also reported that *P. funiculosum* was predominantly associated with core rot of starking apples.

Emericella (represented by *E. nidulans, E. quadrilineata*) and *Paecilomyces* (*P. variotii*) were isolated from four kinds of fruit juices or beverages. *Paecilomyces variotii,* a heat resistant fungus was present in 17 % of mango fruits analysed in Southern Nigeria (Ugwuanyi and Jason 1991).

Acremonium (2 species), *Cladosporium* (2) and *Syncephalastrum* (1) were recorded from three products of which *A. strictu, C. cladosporioides* and *S. racemosum* were the most common. Most of these species were reported previously from different fruit products but with variable incidences (Beuchat 1995 ; Pao et al. 1998).

Other moulds such as *Alternaria alternata, Cochliobolus lunatus* and *Cunninghamella echimulata* were isolated only from two products. Moubasher et al (1971) isolated *A. alternata* from citrus plants in rare frequency, while Combrink et al (1985) isolated it from 59.1 % of discoloured tissue of starking apples. Also, Wojtas-Koziel and Borecka (1990) isolated 206 isolates of *Alternaria* spp. from apple fruits of which *A. alternata* was the most common and the highest number of isolates was pathogenic to apples.

Xerophillic fungi recovered on 20 % sucrose-Czapek agar : Twenty seven species and one variety of each of *A. flavus* and *A. fumigatus* appertaining to 8 genera were collected from the fruit juices and beverages on 20 % Sucrose-Czapek's agar at 28°C. The total counts of viable fungal propagules widely fluctuated between 83-535 colonies/50 ml and the highest count was found in guava juice and the lowest in grape beverages similar to those observed with DRBC medium (Table 3).

Aspergillus (11 species and 2 varieties) and *Penicillium* (8 species) were the most common genera as found to occur in 80-100 % of the samples. Tanaka and Miller (1963) isolated 124 mould isolates from 48 spoiled samples of dried California prunes and noted that *A. niger* was found in 15 % and other *aspergilli* in 4 %, while *Pencillium* comprised 33 % of the total fungi.

Eurotium was the second most common genus and isolated from all types of samples, except for peach beverages. Species of *Eurotium* are known to survive the commercial processing of fruit juices. This ability is due to the production of ascospores in their teleomorphic state (Pitt and Hocking 1985).

Alternaria alternata, A. tenuissima, Cladosporium cladosporioides, Gibberella intricans, Paecilomyces variotii and *Pleospora herbarum* were isolated only from one or two kinds of juices or beverages. All the above species have been isolated from different fruit juices and beverages and other fruit parts as reported by several researchers all over the world (Moubasher et al. 1971; El-Kady et al. 1979; Combrink et al. 1985; Wojtas-Koziel and Borecka 1990).

Natural occurrence of mycotoxins : Thin layer chromatographi analysis of the chloroform extracts of the different fruit juices and beverages clearly showed that all the five apple beverages tested were naturally contaminated with aflatoxins, B_1 and G_1 with concentrations ranging from 20-30 µg/L. Only two of five guava juice samples were also found to be contaminated, but only with aflatoxin B_1 at a concentration of 12 µg/L. All samples of mango juices, grape and peach beverages (5 each) were free from mycotoxins. These findings had certain similarities with earlier studies. Sutic et al (1979)

TABLE 2. TOTAL FUNGAL COUNTS (TFC) AND PERCENTAGE FREQUENCY (%F) OF FUNGAL GENERA AND SPECIES RECOVERED FROM DIFFERENT KINDS OF FRUIT JUICES AND BEVERAGES ON DICLORAN ROSE-BENGAL MEDIUM (DRBC) AT 28°C

Genera and Species	Mango juice beverages		Guava juice beverages		Natural grape beverages		Natural apple beverages		Peach beverages	
	TFC	% F	TFC	% F	TFC	% F	TFC	% F	TFC	% F
Acremonium	5	60	-	-	1	20	1	20	-	-
A. rutilum W. Gams	-	-	-	-	1	20	-	-	-	-
A. strictum W. Gams	5	60	-	-	-	-	1	20	-	-
Alternaria alternata (Fries) Keissler	-	-	-	-	1	20	1	20	-	-
Aspergillus	271	100	414	100	148	100	249	100	319	100
A. flavus Link	55	100	83	100	23	100	40	100	56	100
A. flavus var. columnaris Raper and Fennell	-	-	4	40	2	20	-	-	-	-
A. fumigatus Fresenius	186	100	267	100	106	100	180	100	226	100
A. fumigatus var. *albus* Rai, Tewari and Agarwal	6	80	13	80	1	40	6	60	8	100
A. japonicus Saito	-	-	11	40	-	-	-	-	-	-
A. niger Van Tieghem	19	80	25	80	15	80	16	100	26	100
A. oryzae (Ahlb.) Cohn	1	20	2	20	-	-	-	-	-	-
A. sydowii (Bain. and Sart.) Thom and Church	2	20	-	-	-	-	-	-	-	-
A. tamarii Kita	-	-	1	20	-	-	1	20	-	-
A. terreus Thom	2	40	7	80	-	-	2	40	2	40
A. ustus (Bain.) Thom and Church	-	-	-	-	-	-	2	20	1	20
A. versicolor (Vuill.) Tiraboschi	-	-	1	20	1	20	-	-	-	-
A. zonatus Kwon and Fennell	-	-	-	-	-	-	2	20	-	-
Cladosporium	2	40	-	-	8	60	2	40	-	-
C. cladosporioides (Fres.) de Vries	2	40	-	-	7	60	2	40	-	-
C. herbarum (Pers.) Link ex Gray	-	-	-	-	1	20	-	-	-	-
Cochliobolus lunatus Nelson and Haasis	1	20	-	-	-	-	1	20	-	-
Cunninghamella echinulata (Thaxt.) Thaxt. ex Blakeslee	-	-	-	-	2	20	-	-	3	60
Emericella	2	40	2	40	1	20	-	-	1	20
E. nidulans (Eidam) Vuillemin	-	-	-	1	20	-	-	-	-	-
E. quadrilineata (Thom and Raper) Benjamin	2	40	1	20	1	20	-	-	1	20
Gibberella fujikuroi (Sawada) Ito	2	40	-	-	-	-	-	-	-	-
Mucor hiemalis Wehmer	-	-	1	20	-	-	-	-	-	-
Nectria haematococca Berkeley and Brown	2	40	-	-	-	-	-	-	-	-
Paecilomyces variotii Bainer	2	40	1	20	-	-	1	20	6	60
Penicillium	54	100	99	100	26	100	46	100	52	100
P. aurantiogriseum Dierckx	5	60	-	-	5	20	3	60	2	20
P. brevicompactum Dierckx	-	-	-	-	1	20	1	20	-	-
P. chrysogenum Thom	9	60	8	60	2	20	-	-	1	20
P. citrinum Thom	18	60	-	-	3	20	-	-	-	-
P. corylophilum Dierckx	-	-	-	-	-	-	8	40	27	100
P. duclauxii Delacroix	6	60	74	100	10	60	2	40	12	40
P. funiculosum Thom	15	100	10	40	2	20	14	60	6	60
P. islandicum Sopp	-	-	3	20	3	20	15	100	5	60
P. piceum Raper and Fennel	1	20	-	-	-	-	3	60	1	20
P. verruculosum Peyronel	-	-	4	20	-	-	-	-	-	-
Syncephalastrum racemosum (Cohn) Schroeter	-	-	1	20	-	-	1	20	2	20
Total count	340		518		187		302		384	
Number of genera = 13	9		6		7		8		6	
Number of species = 35 + 2 varieties	19 + 1 var.		18 + 2 var.		17 + 2 var.		20 + 1 var.		16 + 1 var.	

TFC = Total fungal count calculated per 50 ml, product

reported that presence of aflatoxins in fruit juices and vegetables at Serbia (Yugoslavia). Neelakantan et al (1983) examined several fruit samples in Tamil Nadu region (India) and reported that one out of six samples of apple analysed was contaminated with aflatoxin B_1.

Although several samples were contaminated with various toxic fungi, only aflatoxins were detected in the crude extracts of some these samples. This clearly shows that the presence of mycotoxic fungi in a product does not automatically indicate the presence of mycotoxins, especially if growth has not occurred. On the other hand, the toxins may persist long after vegetative growth has occurred and the moulds have died.

With regard to patulin, it was not completely detected in any of the 25 samples tested, whereas numerous investigations

TABLE 3. TOTAL FUNGAL COUNTS (TFC) AND PERCENTAGE FREQUENCY (%F) OF FUNGAL GENERA AND SPECIES RECOVERED FROM DIFFERENT KINDS OF FRUIT JUICES AND BEVERAGES ON 20 % SUCROSE-CZAPERK'S MEDIUM AT 28°C

Genera and Species	Mango juice beverages		Guava juice beverages		Natural grape beverages		Natural apple beverages		Peach beverages	
	TFC	% F	TFC	% F	TFC	% F	TFC	% F	TFC	% F
Alternaria	-	-	-	-	2	40	-	-	-	-
A. alternata (Fries) Keissler	-	-	-	-	1	20	-	-	-	-
A. tenuissima (kunze: Pers.) Wiltshire	-	-	-	-	1	20	-	-	-	-
Aspergillus	222	100	407	100	49	80	91	80	71	100
A. flavus Link	61	100	119	100	24	80	33	80	25	80
A. flavus var. columnaris Raper and Fennell	-	-	-	-	-	-	-	-	1	20
A. fumigatus Fresenius	96	100	199	100	14	40	35	60	2	40
A. fumigatus var. *albus* Rai, Tewari and Agarwal	-	-	16	100	-	-	-	-	-	-
A. niger Van Tieghem	42	80	43	80	5	60	15	60	38	100
A. ochraceus Wilhelm	3	40	-	-	1	20	-	-	-	-
A. oryzae (Ahlb.) Cohn	-	-	-	-	1	20	-	-	-	-
A. parasiticus Speare	-	-	5	20	-	-	-	-	-	-
A. sydowii (Bain. and Sart.) Thom and Church	-	-	-	-	-	-	-	-	1	20
A. tamarii Kita	-	-	-	-	-	-	-	-	2	40
A. terreus Thom	6	40	10	80	3	20	-	-	2	40
A. ustus (Bain.) Thom and Church	-	-	1	20	-	-	-	-	-	-
A. versicolor (Vuill.) Tiraboschi	14	60	14	40	1	20	8	40	-	-
Cladosporium cladosporioides (Fres.) de Vries	5	40	3	20	-	-	-	-	-	-
Eurotium	7	40	2	20	7	20	3	60	-	-
E. amstelodami Mangin	5	20	-	-	-	-	2	20	-	-
E. chevalieri Mangin	-	-	-	-	7	20	1	20	-	-
E. repens De Barry	2	20	2	20	-	-	-	-	-	-
Gibberella intricans Wollenw.	-	-	-	-	-	-	1	20	-	-
Paecilomyces variotii Bainer	-	-	1	20	-	-	-	-	-	-
Penicillium	121	100	122	100	25	80	62	80	17	100
P. aurantiogriseum Dierckx	24	80	25	80	-	-	-	-	1	20
P. brevicompactum Dierckx	-	-	-	-	5	20	-	-	-	-
P. chrysogenum Thom	2	20	7	20	-	-	23	60	11	80
P. citrinum Thom	-	-	-	-	-	-	7	60	4	40
P. duclauxii Delacroix	20	40	-	-	-	-	-	-	-	-
P. funiculosum Thom	55	60	14	80	20	80	32	80	-	-
P. islandicum Sopp	20	80	31	20	-	-	-	-	1	20
P. oxalicum Currie and Thom	-	-	45	80	-	-	-	-	-	-
Pleospora herbarum (Fr. Rabenh. Ex Ces. and de Not.)	2	20	-	-	-	-	-	-	-	-
Total count	357		535		83		157		88	
Number of genera = 8	5		5		5		5		2	
Number of species = 27 + 2 varieties	15		15 + 2 var.		11		9		10 + 1 var.	

TFC = Total fungal count calculated per 50 ml, product

have reported its presence as the main myctoxin in fruit juices especially apple juice (Brackett and Marth 1979; Bata et al. 1983). In the present work, thin layer chromatographic analysis was done and this method was found suitable only for samples with high patulin content (above 50 µg/Kg) as mentioned by Bata et al (1983). In many countries, the maximum permissible patulin content for apple pulps is 20-30 µg/Kg. Brackett and Marth (1979) examined 20 samples of apple juices in Wisconsin (USA) and reported that most samples contained less than 50 µg of patulin per litre. Bata et al (1983) found the patulin concentration in apple juice to range from zero to 13 µg/kg in Budapest (Hungary).

Also, citrinin, diacetoxyscirpenol, ochratoxins, rubratoxins, sterigmatocystin, T-2 toxin and zearalenon were not detected in any sample of fruit juices or beverages tested.

In conclusion, from the mycological analyses of the juice and beverage samples, it was revealed that all the samples were contaminated with a large variety of fungal species, but low in their numbers. The most contaminated samples were those of guava juices and the lowest were those of grape beverages. Also, it was found that *A. flavus*, *A. fumigatus* and *A. niger* were recovered from all samples of fruit juices and beverages. *Penicillium funiculosum* was common in all samples. TLC analysis of the chloroform extracts of the five samples of each of mango juice, grape and peach beverages showed no detectable levels of mycotoxins. However, the five apple beverage samples contained aflatoxins B_1 and G_1 at concentrations of about 20-30 µg/L. Also, two (out of five)

guava samples contained, aflatoxin B_1 at 12 µg/L. The detection of such toxins in apple and guava juices is considered to be first record in Egypt, which can threaten human health. In this background, concepts of good manufacturing practices and hazard analysis critical control points are to be implemented in the fruit juices and beverages industry.

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Studies on Storage and Dehydration Characteristics of Onion Cultivars

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Seven cultivars of onions were stored in nylon-netted bags at room temperature for 2 months and storage losses were studied. The losses due to rotting were found to be maximum in 'Decco' cultivar, whereas, minimum was in 'Parbhani Local' followed by 'Tadola Local', 'Pusa Selection' and 'Red Creole'. On dehydration of 3 mm thick rings of onion in an air cabinet drier (55±2°C for 9 h with air flow rate of 1.46 m/sec and loading of 4.3 kg/m²) to 5-6% (wet basis) moisture content, the 'Jalgaon White', 'Phule White', 'Decco' and 'Red Creole' were found suitable for dehydration with better physico-chemical and organoleptic qualities. The extent of loss of pungency during dehydration ranged from 67.3 to 74.4%. 'Decco' cultivar retained maximum pungency, followed by 'Red Creole'. Organoleptically, the white cultivars scored maximum for all the parameters as compared to the red at fresh, dehydrated and rehydrated forms.

Keywords : Onion cultivars, Storage, Dehydration, Rehydration, Pungency, Organoleptic evaluation.

Onions are highly perishable and therefore, high post-harvest losses of this commodity occur during transportation and storage. The losses of onions in storage have gone upto 40% annually (Pandey 2000). Most of these losses are attributed to inadequate and improper storage infrastructure, transport and marketing facilities. Therefore, the cost of onions fluctuates from season to season and year to year. The demand for fresh and dehydrated onion has considerably increased over the last 2 decades (Raina et al. 1988). Dehydration is one of the feasible methods of preservation of surplus produce, which will minimize the fluctuation in market price during storage. Dehydrated onions are the products of considerable importance in world trade. Since, this is a highly priced commodity in the international market, a proper selection of suitable cultivar of onion is very essential to meet the international standards of quality at competitive rates (Sethi et al. 1973).

To meet this challenge, many new cultivars possessing superior field characteristics are being developed. However, their storage and rehydration characteristics differ significantly. The Indian cultivars, generally, have low solids, less pungency and shortened shelf life, which limit their suitability for dehydration (Sharma and Nirankarnath 1991). Some studies on suitability of dehydration characteristics of some cultivars of onions have been carried out (Maini et al. 1984; Singh and Mohan Kumar 1984; Raina et al. 1988). The present study was designed to study the storage and dehydration characteristics of 5 white and 2 red cultivars, which have not been studied so far.

Mature and cured bulbs of 5 white cultivars ('Jalgaon White', 'Phule White', 'Decco', 'Pusa Selection' and 'Tadola Local') and 2 red cultivars ('Red Creole' and 'Parbhani Local') under the field trials at the Department of Horticulture, College of Horticulture, Marathwada Agricultural University, Parbhani were obtained.

Evaluation of physico-chemical characteristics : The average weight, bulb size, shape index, colour and nature of bulb neck were determined on 10 bulbs of each cultivar, which were selected at random. The moisture, ash, total soluble solids (TSS) and total solids (TS) of each cultivar were determined by the methods of Ranganna (1986).

The pungency in terms of pyruvic acid (PA) was evaluated by the method of Schwimmer and Weston (1961). The physiological losses in weight, sprouting, rotting, peeling and trimming losses were recorded by weighing the onions after 2 months of storage.

Dehydration of onions : Bulbs were peeled, trimmed and sliced manually into 3 mm thick rings. The cultivars were dehydrated without any pre-treatment. Rings were spread thinly on trays (tray loading 4.3 kg/m² with a air flow rate of 1.46 m/sec) and dehydrated in an air cabinet drier (55 ± 2°C, about 9 h) to 5-6% (wet basis) moisture content. Dehydrated rings were packed quickly in polythene bags and stored in desiccators for further studies. The dehydration characteristics such as yield, dehydration ratio and rehydration ratio were determined by using standard procedures (ISI 1967). The organoleptic evaluation of fresh, stored, dehydrated and rehydrated onion rings was carried out by a panel of semi-trained judges with respect to colour, texture, taste, pungency and overall acceptability using a 9-point Hedonic scale. The data were analyzed by using completely randomized block design as per the method of Panse and Sukhatme (1967).

Physico-chemical characteristics : The data pertaining to physico-chemical characteristics are presented in Table 1. The average bulb weight was the highest in 'Jalgaon White', followed by 'Phule White', 'Decco', 'Pusa Selection', 'Red Creole' and 'Tadola Local', while it was minimum in 'Parbhani Local'. The average bulb diameter of all cultivars was higher than length except 'Decco'. The shape of the 'Decco' cultivar was oblong, whereas, the shapes of other cultivars were flat globe. Generally, bulbs with 5-6 cm diameter with full globe shape are preferred for dehydration to facilitate handling in slicing machine. Except 'Decco', all other cultivars had bulb diameters between 5-6 cm with flat globe shapes and hence, were suitable for dehydration. Among all cultivars, 'Decco' showed a significant difference in shape index with open neck.

The lower moisture and significantly higher TSS and TS contents were observed in 'Red Creole' and 'Decco', followed by 'Pusa Selection', 'Jalgaon White', 'Parbhani Local', 'Tadola Local' and 'Phule White' in that order. Raina et al (1988) reported that 11.0 to 14.8% TSS were most suitable for dehydration of onions. The data presented in Table 1 indicated that all the 7 cultivars studied were suitable for dehydration on the basis of TSS and TS contents. The total ash contents of all these cultivars for deciding their suitability for dehydration

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TABLE 1. PHYSICO-CHEMICAL CHARACTERISTICS OF ONION CULTIVARS BEFORE STORAGE

Parameters	Cultivars 'Jalgaon White'	'Phule White'	'Decco'	'Pusa Selection'	'Tadola Local'	'Parbhani Local'	'Red Creole'	CD at 5%
Skin colour	White	White	White	White	White	Light red	Red	
Average bulb weight, g	95.50	91.90	83.40	79.80	73.20	48.90	73.50	16.300
Average bulb size, L x B* cm	4.84 x 6.08	5.11 x 6.16	8.39 x 4.67	4.69 x 5.75	4.99 x 5.32	4.73 x 5.04	5.16 x 5.54	
Shape index	0.80	0.84	1.89	0.81	0.93	0.94	0.93	0.198
Bulb neck	Closed	Closed	Open	Closed	Closed	Closed	Closed	
Moisture, %	85.50	85.70	82.90	86.30	87.10	86.50	82.30	0.840
TSS, °Brix	12.50	11.60	15.10	12.60	12.20	12.40	15.20	0.670
Total solids, %	14.46	14.28	16.94	13.66	13.06	13.46	17.72	0.840
Total ash, %	0.57	0.63	0.64	0.54	0.64	0.44	0.72	0.012

*L = Length (cm), B = Breadth (cm)
Each figure is the average of 10 bulbs of each cultivar

were also in the range of 0.44 to 0.77% as reported by Kalra et al (1986).

The data on post-harvest losses, pungency and TSS of onion cultivars on 2 months storage (Table 2) showed that rotting and sprouting losses in 'Decco' cultivar were 60 and 30%, respectively and had the highest physiological loss in weight, thereby indicating that, this cultivar was not suitable for dehydration. The physiological weight loss was maximum (16.94%) in 'Decco', whereas, it was minimum (7.83%) in 'Red Creole'. Maini et al (1984) reported 7.5 to 18.2% physiological weight losses in onions after storage for 2 months. The data in the present investigation fairly coincided with those of Maini et al (1984). As compared with other cultivars, 'Pusa Selection' and 'Red Creole' were found better with respect to rotting and physiological weight losses and therefore, could be considered for storage and subsequent dehydration. Pungency (PA) of onions is an important trait and imparts a characteristic flavour during dehydration. This was found to be highest in 'Decco' and lowest in 'Parbhani Local'. The TSS contents during storage (Table 2) indicated that 'Red Creole', 'Parbhani Local' and 'Pusa Selection' cultivars were better for storage with respect to % change in TSS, indicating that there were least moisture losses from these cultivars during storage.

TABLE 2. PHYSICO-CHEMICAL CHARACTERISTICS OF ONION CULTIVARS (AFTER 2 MONTHS STORAGE AT ROOM TEMPERATURE)

Cultivars	Rotting loss, %	Sprouting loss, %	Physio-logical loss in weight, %	Pyruvic acid, µmol/g	TSS, °Brix
'Jalgaon White'	25	–	12.11	81.52	13.2
'Phule White'	35	–	12.20	79.68	12.7
'Decco'	60	30	16.94	89.63	15.6
'Pusa Selection'	20	–	8.30	76.30	12.8
'Tadola Local'	15	–	10.53	71.62	12.5
'Parbhani Local'	10	–	8.02	59.84	12.8
'Red Creole'	20	–	7.83	62.90	15.8
CD at 5%	–	–	N.S.	–	0.56

NS = Not significant
Each figure is the average of 10 bulbs of each cultivar

The technical data on dehydration characteristics of onion rings depicted in Table 3 indicate maximum peeling and trimming losses in 'Tadola Local', followed by 'Decco'. The losses in other cultivars, however, were not significant. The oblong neck character and thick root disc in 'Decco' and 'Tadola Local' led to more peeling and trimming losses. Raina et al (1988) reported peeling and trimming losses ranging from 2.2 to 3.5% on storage of different cultivars of onions for 12 months. However, high peeling and trimming losses in the present study may probably be due to varietal differences. The yield of dehydrated onion rings was found maximum (17.5%) in 'Red Creole' and minimum (15.2%) in 'Jalgaon White' with intermediate values in rest of the cultivars. Variations in the yields of dehydrated onion rings were basically due to more TSS and TS contents. Raina et al (1988) reported positive correlationship between the TSS and yield, agreeing with the data of the present investigation. The dehydration ratio is a commercially important criterion for dehydration of onions. Lower the dehydration ratio, better is the

TABLE 3. TECHNICAL DATA ON DEHYDRATION OF ONION CULTIVARS

Cultivars	Peeling and trimming loss, %	Dehydration yield, %	Dehydration ratio	Rehydration ratio	Final moisture, %	Pyruvic acid, µmol/g	Loss in PA, %
'Jalgaon White'	8.00	15.20	6.6:1	1:4.67	4.80	23.10	71.60
'Phule White'	8.50	16.40	6.1:1	1:5.00	5.00	21.90	72.50
'Decco'	10.00	15.62	6.4:1	1:4.50	4.90	29.30	67.30
'Pusa Selection'	8.00	15.27	6.6:1	1:5.00	4.90	22.70	70.20
'Tadola Local'	12.00	15.81	6.3:1	1:5.33	4.73	18.93	73.60
'Parbhani Local'	8.10	15.25	6.6:1	1:5.00	4.93	15.32	74.40
'Red Creole'	8.40	17.53	6.1:1	1:4.66	4.80	19.78	68.40
CD at 5%	–	0.167	–	–	0.225	–	–

Each figure is the average of 10 bulbs of each cultivar

TABLE 4. ORGANOLEPTIC EVALUATION OF FRESH, DEHYDRATED AND REHYDRATED ONION CULTIVARS

Cultivar		Organoleptic qualities*				
		Colour	Texture	Taste	Pungency	Overall acceptability
'Jalgaon White'	F	2.77	2.82	2.76	2.89	2.91
	D	2.89	2.91	2.73	2.80	2.94
	R	2.84	2.84	2.70	2.86	2.91
'Phule White'	F	2.87	2.82	2.72	2.82	2.78
	D	2.78	2.78	2.63	2.71	2.78
	R	2.80	2.78	2.78	2.84	2.80
'Decco'	F	2.89	2.82	2.68	2.89	2.81
	D	2.87	2.87	2.79	2.89	2.94
	R	2.91	2.77	2.77	2.77	2.82
'Pusa Selection'	F	2.92	2.92	2.73	2.90	2.89
	D	2.44	2.67	2.38	2.57	2.75
	R	2.71	2.69	2.69	2.67	2.77
'Tadola Local'	F	2.74	2.80	2.62	2.78	2.78
	D	2.59	2.69	2.48	2.53	2.64
	R	2.66	2.71	2.80	2.71	2.73
'Parbhani Local'	F	2.72	2.84	2.59	2.76	2.82
	D	2.39	2.65	2.50	2.60	2.62
	R	2.64	2.75	2.73	2.80	2.79
'Red Creole'	F	2.78	2.77	2.66	2.72	2.65
	D	2.69	2.63	2.65	2.57	2.73
	R	2.80	2.84	2.69	2.71	2.80
CD at 5%	F	N.S.	N.S.	N.S.	N.S.	0.187
	D	0.174	0.176	0.162	0.187	0.127
	R	0.125	N.S.	N.S.	0.118	N.S.

* = Mean of square root of x + 0.5
F = Fresh, D = Dehydrated, R = Rehydrated
NS = Not significant
Each figure is the average of 10 bulbs of each cultivar

yield of finished product. In the present study, white coloured onion cultivars exhibited lower dehydration, which could probably be due to more TS. However, 'Red Creole' was observed to be an exception to this characteristic. As expected and reported by Singh and Mohan Kumar (1984) that lower the dehydration ratio, better was the yield of finished product. The ratio was observed to be from 6.1:1 to 6.6:1, which was fairly comparable to that reported by Singh and Mohan Kumar (1984). Similarly, the rehydration ratio is also one of the important factors in the dehydrated product. It was observed to be more or less similar to the standard specifications laid down by Fruit Products Order (FPO 1955) for onion. It was highest in 'Tadola Local' and lowest in 'Decco' with intermediate values for rest of the cultivars. The data on this characteristic were fairly comparable with those of Kalra et al (1988) and Singh and Mohan Kumar (1984). The moisture contents however, significantly changed in all the cultivars studied and also similar to those of Kalra et al (1986). The pungency (PA) of onions is also considered as an important trait for dehydration, which must be retained in the final product for better flavour. The pungency retained in 'Decco' cultivar was maximum (29.3 µmol/g), whereas, minimum (2.3 µmol/g) in 'Parbhani Local'. In general, the red coloured cultivars retained more pungency than white coloured cultivars except 'Tadola Local'.

From the above data, it may be inferred that, 'Decco' among white cultivars and 'Red Creole' among red cultivars were most suitable for dehydration due to their better capacity of pungency retention. The loss of pungency usually occurs during dehydration, which may be due to heat and other degradative changes in PA. The losses also occurred in PA in all the cultivars under study and were comparable with the data reported earlier (Ismail 1977; Raina et al. 1988; Sharma and Nirankamath 1991).

The data on organoleptic evaluation of the onions, while fresh, after dehydration and rehydration as reported by a panel of semi-trained judges presented in Table 4 indicated that, some of the white cultivars were better for these characteristics when fresh, whereas, few others were better in these characteristics after dehydration and rehydration. The cultivars 'Jalgaon White' and 'Pusa Selection' scored maximum for overall acceptability, pungency, taste and texture in fresh condition. On dehydration, however, the cultivars 'Decco' and 'Jalgaon White' scored maximum for overall acceptability, colour, taste and texture, which were indicative of their better suitability for dehydration. A slight different trend of these characteristics was observed in these cultivars on dehydration. The 'Red Creole' was better on the basis of maximum score obtained for texture, whereas, 'Jalgaon White' was better with respect of pungency, colour and texture. The overall acceptability score was maximum for 'Decco' and 'Jalgaon White' cultivars after rehydration.

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RESEARCH NOTE

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Effect of Dextran on the Rheological Behaviour of Indian Molasses

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The effect of addition of dextran on the apparent viscosity of molasses was studied, using a Brookfield Rheometer. The apparent viscosity was found to increase with the addition of dextran and decrease with increase in rpm. The behaviour observed with and without dextran was pseudoplastic. The apparent viscosity of molasses was found to depend on temperature. The dependence of apparent viscosity on temperature was related through the Arrhenius law. The activation energy observed was in the range of 2.237 - 2.482 kcal/g. mol.

Keywords : Dextran, Molasses, Activation energy, Pseudoplastic, Apparent viscosity.

The viscosity of molasses varies with the nature and amount of non-sucrose present in it, like the inorganic salts, macromolecules, invert sugar etc (Kumar et al. 1994). The viscosity increases rapidly with increase in the total solids (Payne 1959). It roughly doubles with an increase of 0.8% in total solids. An extensive survey has been done on the flow behaviour of molasses. It has been generally observed that cane and beet molasses have been found to show non-Newtonian and Newtonian behaviour, respectively. Kumar et al (1994) observed the behaviour of Indian molasses to be non-Newtonian at low shear rates. Broadfoot et al. (1990) studied the rheological behaviour of massecuites and molasses from Australian sugar factories and found them to show pseudoplastic behaviour but their viscosity values varied widely as did their composition. However, they showed a high linear correlation co-efficient for the dependence of viscosity on temperature in accordance with Ostwald law. Toledo (1991) has indicated a shear thinning behaviour of molasses. Thus, it appears that the rheological behaviour of two molasses depends upon the source, environmental condition of the cane crop, extent of maturity etc. The study of rheological behaviour of molasses is important, as it provides information on its physical properties and helps in understanding the underlying mechanisms of momentum and heat transfer process. Thus, it becomes necessary to investigate and study the rheological properties of individual components present in molasses so as to establish their classification.

Mechanical harvesting has resulted in an increase in the quantity of bacterial polysaccharides like soluble dextran in the juice (Cuddihy 1999). Dextran that enters the juice remains with it till crystallization of sugar. Dextran formation causes sugar loss (Donal 1994), processing problems (James et al. 1971), increases the juice viscosity manifold (Sikdar et al. 1979), which decreases the clarification rate and gives poor clarification, leads to crystal elongation (Covacevich et al. 1977, VanHook 1981) and also hinders the heat transfer process. Mantovani et al (1967) have reported that dextran hinders the crystallization process by a temporary adsorption on, especially the glucose fructose portion of the molecule. Cuddihy (1999) has reported sugar loss between 1.2 and 1.4 purity points for every 1000 ppm/Brix of dextran in molasses. The effect of addition of dextran on the viscosity of molasses is thus an important parameter to be known for understanding its behaviour. Literature has no information on its rheological behaviour. The present study was, therefore, undertaken to study the effect of addition of dextran up to the extent present in juices on the rheological properties of molasses.

Molasses sample from Rana Sugar Mills, Buttari of 85 Brix and 32.4 purity served as a base material. The Brix was determined using the Abbemat Digital Automatic Refractometer, while purity which is the ratio of pol to Brix was determined using an Automatic Sucromat. (Dr. Wolfgang Kernchen GMBH, Seelze, Germany). Dextran of CDH laboratory grade was blended with molasses up to the maximum of 1000 ppm/Brix. Viscosity determination of molasses samples containing dextran was conducted with Brookfield HBDV (III) Programmable Rheometer (Brookfield Eng. Lab. Inc Stoughton MA). Disc Spindles HB2, HB3, HB4 and HB5 were used for sample measurement to cover the entire concentration range and apparent viscosity was determined as a function of rpm. For the control of temperature, a Brookfield thermostatic bath (TC-500) supplied along with the instrument was used. The sample was kept in a water bath for about half an hour for equilibrium to be attained before the reading was recorded. Studies were conducted by stepwise addition of varying amounts of dextran to molasses up to a total of 60 g in 600 g molasses taken in a 500 ml beaker with flat bottom of diameter 8 cm. The rpm was set to get the torque with a given spindle between 10 and 90 Nm.

The effects of addition of dextran on the apparent viscosity of molasses at 50C are shown in Fig. 1, 2, 3 and 4. The viscosity of molasses was found to increase with increase in the concentration in dextran. The apparent viscosity, however, decreased with increase in rpm from 10 to 150 s^{-1}, thereby indicating a shear thinning behaviour. The viscosity of pure molasses was observed to range between 2 and 3.3 Pa.s. and increase up to 100 Pa.s with the addition of dextran. The behaviour observed was pseudoplastic specifically at low shear rates. Fig. 5 illustrates the exponential rise in apparent viscosity at 30 rpm on the addition of dextran.

Effect of temperature on apparent viscosity : The rheological parameters for dextran addition (30 g and 60 g) to molasses were studied between 30 and 55°C and in the rpm range between 1 and 100 s^{-1} for dextran addition to molasses. However, at the rpm of 7 s^{-1} and 10 s^{-1} for which the data were

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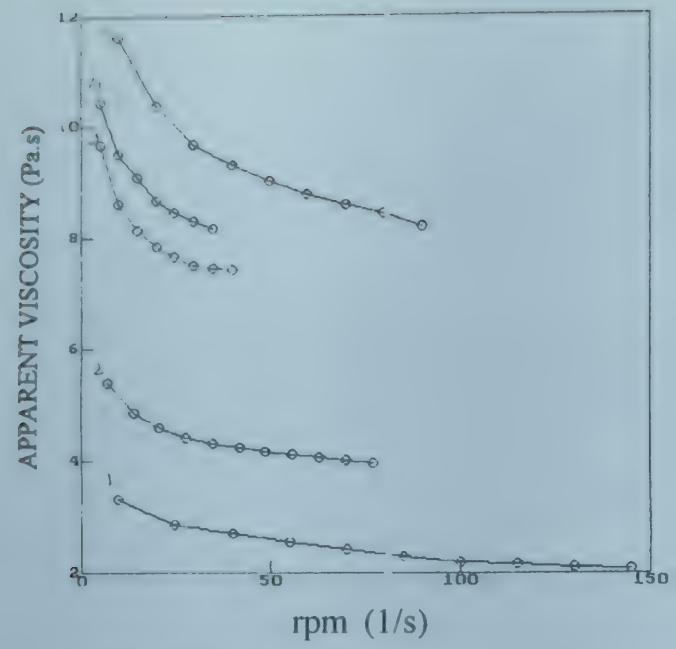

Fig. 1. Apparent viscosity vs rpm using spindle HB2 (1) pure molasses (2) 1 g dextran + molasses (3) 2 g dextran + molasses (4) 3 g dextran + molasses (5) 4 g dextran + molasses

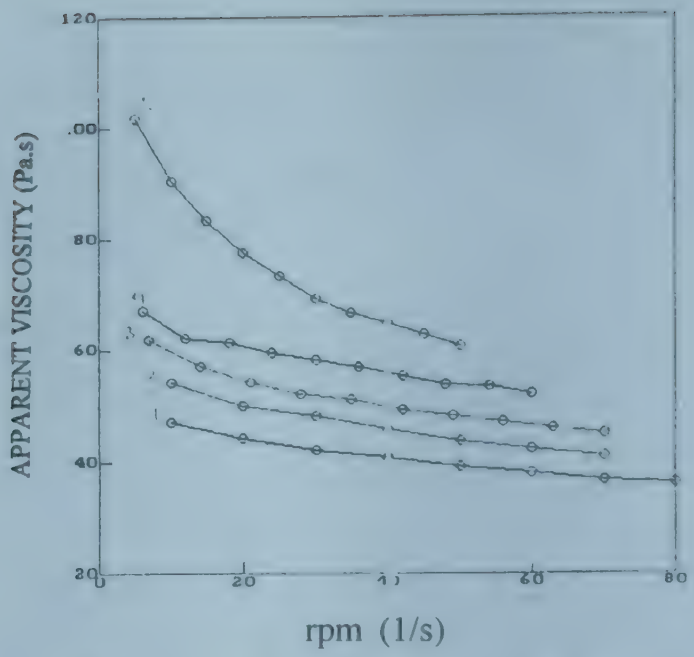

Fig. 4. Apparent viscosity vs rpm using spindle HB5 (1) 48 g dextran + molasses (2) 51 g dextran + molasses (3) 54 g dextran + molasses (4) 57 g dextran + molasses

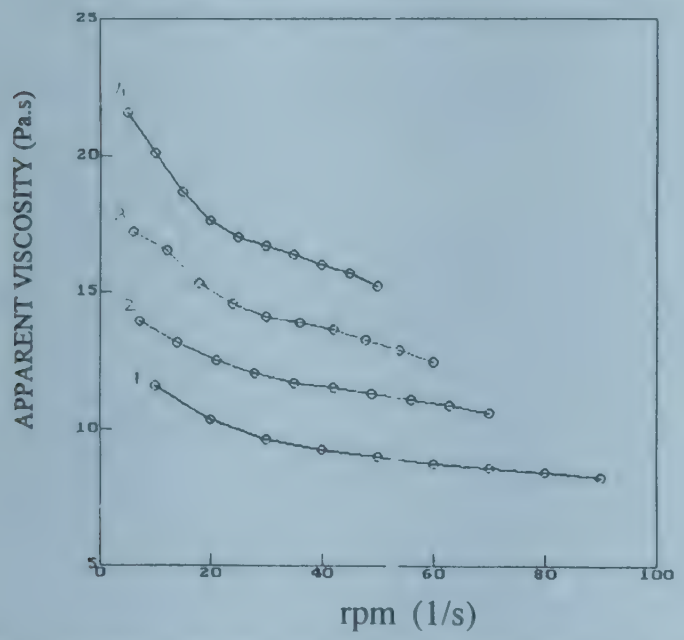

Fig. 2. Apparent viscosity vs rpm using spindle HB3 (1) 6 g dextran + molasses (2) 12 g dextran + molasses (3) 18 g dextran + molasses (4) 24 g dextran + molasses

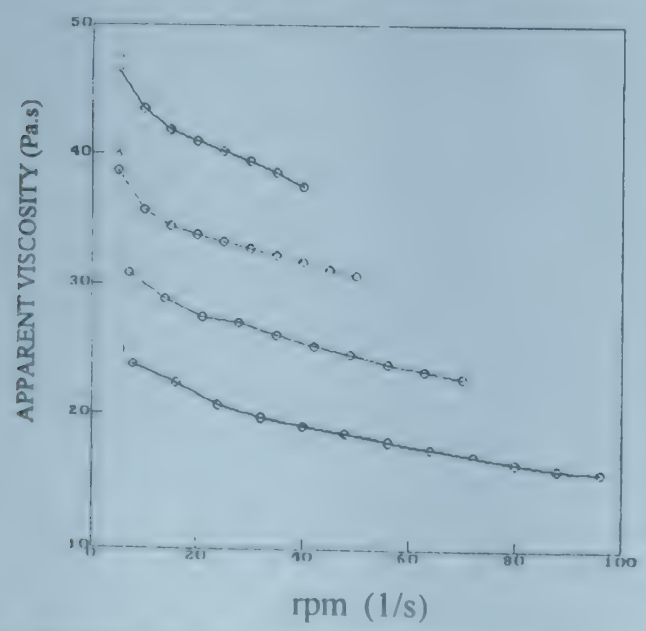

Fig. 3. Apparent viscosity vs rpm using spindle HB4 (1) 27 g dextran + molasses (2) 33 g dextran + molasses (3) 39 g dextran + molasses (4) 45 g dextran + molasses

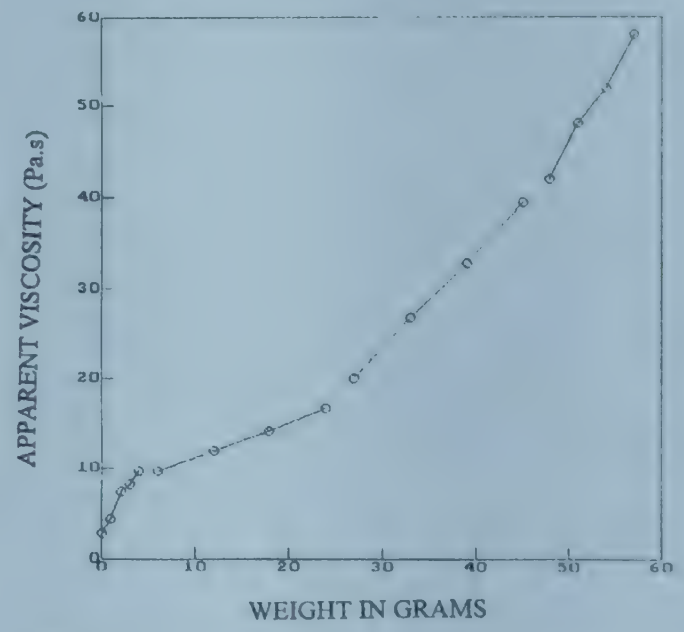

Fig. 5.. Effect of dextran addition on apparent viscosity at 30 rpm

available at all temperatures, the temperature dependence of the apparent viscosity was determined by the Arrhenius model (Eqn. 1).

$$\eta = A \exp(-E_a/RT) \quad \text{------- (1)}$$

where A = Arrhenius constant, R = universal gas constant, T = absolute temperature and E = energy of activation. Eqn. 1 was linearised to give

$$\ln \eta = \ln A - E_a/RT \quad \text{------- (2)}$$

The coefficients of equation (2) were computed using least square regression analysis. The computed values of energy of activation along with the coefficient of correlation and the standard error are presented in Table 1. The high correlation coefficients confirmed the linear relationship. The standard error values were less than 0.044. The activation energy was found to decrease with an increase in rpm and increase with an increase in the dextran level. The plots of apparent viscosity against 1/T (K) for 30 and 60 g of molasses and at 7 and 10 rpm are illustrated in Fig. 6. It has been observed that apparent

TABLE 1. LEAST SQUARE REGRESSION VALUES OF EQN (2) GIVING THE ENERGY OF ACTIVATION, STANDARD ERROR AND CORRELATION COEFFICIENT

Dextran level, g	Rpm s^{-f}	Energy of activation, Kcal/mole	Standard error σ	Correlation coefficient, r
30	7	2.258	0.02943	0.99773
	10	2.237	0.01469	0.99933
60	7	2.482	0.03664	0.99609
	10	2.478	0.02959	0.99741

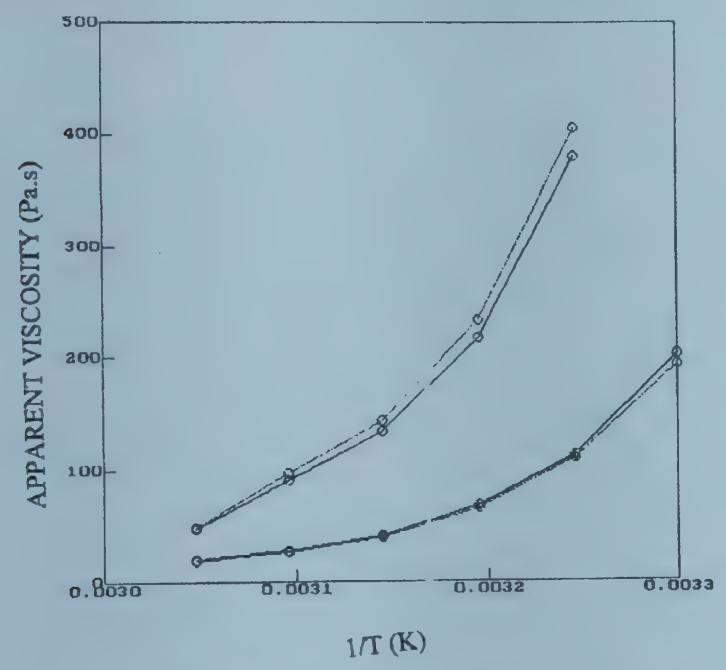

Fig. 6. Effect of temperature on apparent viscosity (1) 30 g dextran 10 rpm (2) 30 g dextran 7 rpm (3) 60 g dextran 10 rpm (4) 60 g dextran 7 rpm

viscosity was found to nearly double with every 5° decrease in temperature. The data indicated that the variation of η with temperature followed the Arrhenius model.

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RESEARCH NOTE

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Mycobiota and Potential Mycotoxins of Date Fruit

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Mould growth has detrimental effects on the quality of foods and may result in mycotoxin contamination. Mycoflora of date fruits collected from different geographical regions of Andhra Pradesh, India was analyzed. The pattern of distribution of fungal population was different both in quality and quantity on different date fruit samples. Species of *Aspergillus*, *Penicillium* and *Fusarium* were dominant mycotoxigenic fungi. The percentage of toxigenicity not only varied with the fungus but also with the sample and geographical conditions.

Keywords : Date fruit, Mycoflora, Mycotoxins.

Moulds are widely distributed in the environment (Jarvis 1983). Mould contamination not only causes deterioration of foods and feeds but also can adversely affect the health of humans and animals, since they may produce toxic metabolites called mycotoxins (Sweetney and Dobson 1998). Especially, human poisoning caused due to consumption of fungal metabolites contaminated foodstuff has been a matter of serious concern all over the world. Natural contamination of agricultural produce with mycotoxins is fairly high in developing tropical countries like India, because of socio-economic backwardness and faulty storage conditions. Among dry fruits, date fruits (*Phoenix dactylifera* L.) are cultivated mostly in the dry regions of Gujarat, Rajasthan, Punjab, Uttar Pradesh, Andhra Pradesh and Karnataka, India. Date fruits are rich in sugars, vitamins and eaten fresh or dried. They are widely used in bakery, confectionery, jams and even in brandy preparation. Though the incidence of mycotoxin producing fungi on other dry fruits is reported (Giridhar and Reddy 1997, 1998), such information is not available on mycotoxigenic fungi associated with date fruits. In this communication, the mycoflora of date fruits (*Phoenix dactylifera* L) in relation to mycotoxin production is presented.

Samples of date fruit (dry form) were collected from different regions of Andhra Pradesh in sterilized polythene bags and condition of the samples and details of storage place were recorded. The mycoflora of date fruits was analyzed using humid chamber method and dilution plate method (Waksman 1922). The fungi were isolated and identified with the help of available literature (Barnett and Hunter 1972; Domesch et al. 1980; Frisvad and Filtenberg 1983). The percentage of frequency of each fungus was calculated. The fungi isolated were screened for their mycotoxin producing potential by TLC as suggested by Scott et al (1970), Udagawa et al (1978), Kamimura et al (1981) and Rao et al (1985). Mycotoxins like citrinin, terreic acid, patulin, cyclopiazonic acid and penitrem were further confirmed as suggested earlier (Damodaran et al. 1973; Subramaniyan 1982, Subramaniyan et al. 1972; Hou et al. 1970, Rathinavelu and Shanmugasundaram 1984). The standard mycotoxins for this study were obtained from Sigma. USA.

Table 1 reveals that there was fairly high incidence of mould infestation on samples from Andhra (95.20), followed by Telangana (82.35) and Rayalaseema (50.00). A total of 51 fungal species representing 21 genera were associated with date fruit samples of different geographical regions of Andhra Pradesh. The percentage of mycotoxigenic fungi positive samples were relatively high in Andhra region. This may be attributed to the warm, humid environmental conditions of that region. The most prominent fungi in different samples comprised species of *Aspergillus, Fusarium* and *Penicillium, Rhizopus stolonifer, Alernaria dianthi*. A perusal of Table 2 reveals the rate of incidence of other non-mycotoxygenic and toxigenic moulds on date fruits.

The aflatoxin and gliotoxin producing potential of *A. flavus* and *A. fumigatus* respectively was highest, followed by *A. ochraceus* for ochratoxin A and *P. citrinum* for citrinin production. The toxigenic potential of trichothecene group mycotoxins produced by *Fusarium* spp was low. In the present study, the isolation and identification of different species of *Penicillium* and the type of mycotoxin produced by it is further identified as suggested by Frisvad and Fltenberg (1983). A range of fungal species from the genus *Aspergillus* and *Penicillium* are able to produce ochratoxin A, which is very common in foods. Unlike aflatoxins, the organisms, which produce ochratoxin A can

TABLE 1. AREAS SURVEYED AND PERCENTAGE OF MOULD INFESTED SAMPLES

Region	Number of districts	Number of samples collected	Number of mouldy samples	% of mouldy samples	% of toxigenic fungi infested samples	% of moisture content of samples	Load of moulds (cfu/g) on samples
Andhra	8	21	20	95.20	80.00	30 - 42	24 - 40
Telangana	8	17	14	82.35	64.28	30 - 39	14 - 28
Rayalaseema	3	4	2	50.00	50.00	30 - 35	8 - 19

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TABLE 2. MYCOFLORA AND MYCOTOXINS OF DATE FRUIT (*PHOENIX DACTYLIFERA*. L)

Name of the fungus	% of frequency	Number of strains screened	Number of toxigenic strains	Mycotoxin*
Alternaria alternata	22.22	12	4	Ateenuene
Aspergillus flavus	40.00	25	13	Aflatoxin B_1 (5-16 ppm)
Aspergillus fumigatus	40.00	28	14	Gliotoxin
Aspergillus nidulans	4.44	4	1	Sterigmatocystin
Aspergillus ochraceus	28.88	16	5	Ochratoxin A
Aspergillus parasiticus	8.88	8	3	Aflatoxin B_1 (4-13 ppm)
Aspergillus terreus	28.88	18	6	Patulin (4-11.5 ppb, 0.01 O.D. = 1 ppb)
			8	Terreic acid (3-10 ppb, 0.01 O.D. = 1 ppb)
Fusarium sp.	15.55			
F. moniliforme		7	4	Diacetoxyscirpenol (32-51 µg/ml)
			2	T-2 toxin (16-18 µg/ml)
			5	Zearalenone (45-62 µg/ml)
F. oxysporum		5	3	Zearalenone (36-49 µg/ml)
			1	Diacetoxyscirpenol (20 µg/ml)
			1	T-2 toxin (14 µg/ml)
F. solani		2	1	T-2 toxin (10 µg/ml)
Penicillium sp.	37.77			
P. citrinum		17	7	Citrinin (18-38 µg/ml)
P. griseofulvum		4	1	Cyclopiazonicacid (6-9.6 µg/ml)
P. oxalicum		3	1	Ochratoxin A
P. verruculosum var verrucosum		2	1	Ochratoxin A
P. purpurogenum		4	1	Secalonic acid D
			1	Ochratoxin A
P. aurantiogrisum		4	1	Penitrem (6-10.4 µg/ml)
Myrothecium roridum	4.44	4	1	Roridin (28-152 µg/ml)
Stachybotrys atra	11.11	8	5	Satratoxin (35-161 µg/ml)
Trichoderma viride	22.22	15	5	Gliotoxin
			3	Trichodermin (10-18 µg/ml)

% of frequency of other fungi : *Absidia corymbifera (14.63), Acremonium terricola (13.30), Alternaria dianthi (22.22), Aspergillus candidus (2.22), A. chevalieri (20.00), A. flavipes (15.55), A. japonicus (13.33), A. niger (46.66), A. sydowii (15.55), A. ustus (28.88), A. versicolor (26.66), Aspergillus* sp. *(20.00), Chaetomium globosusum (4.44), Cladosporium chlorocephalum (8.88), C. cladosporioides (26.66), Curvularia clavata (2.22), C. eragrostoides (2.22), C. lunata (28.88), C. pallescens (13.33), Drechslera hawaiensis (11.11), D. spicifer (13.33), Memnoniella echinata (13.33), Mucor varians (6.66), Paecilomyces varioti (13.33), Penicillium funiculosum (6.66), Phoma sorghina (4.44), Rhizopus stolonifer (33.33), Scopulariopsis brevicaulis (17.77), Syncephalastrum racemosum (17.77), Trichothecium roseum (6.66), Sterile mycelia (13.33).*

*only qualitative data otherwise mentioned quantitative data are in parenthesis.

thrive in the temperatures and humidity, commonly found in temperature climates (MAFF 1999). The filamentous moulds most commonly found in stored agricultural produce are *Aspergillus* and *Penicillium* species, several of which are well-known mycotoxin producers (Sweetney and Dobson 1998). More than 150 *Aspergillus* species have been found, with 45 species being mycotoxin producers (Frisvad and Samson 1991). Similarly, *Penicillium* species are commonly involved in food spoilage and almost all of them produce mycotoxins (Frisvad and Samson 1991). Although many *Fusarium* species exist in nature, only 24 species have been associated with mycotoxin production. In the samples that were analyzed for natural incidence of mycotoxigenic fungi, the incidence of *A. ochraceus* was almost equal to *A. flavus*.

It is of great concern that many species of toxigenic fungi were isolated from date fruits during storage conditions. Date fruits especially collected from warm and humid climates of Andhra Pradesh may be of high risk because of gliotoxin, aflatoxin B_1, ochratoxin A etc. Although the moulds are usually killed in the preparation of foods (e.g., cooking, baking etc.,) an improper processing condition may enable their survival, followed by mycotoxin production in foods. Furthermore, during mixing of food ingredients date fruits can serve as a source of mold contamination of the atmosphere of processing establishment, leading to a recontamination of products after processing. The tendency of mycotoxins to occur in comparatively low concentration may aggravate the problem, when more than one mycotoxin elaborated in the date fruits under faulty storage conditions due to their high sugar content.

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EVALUATION OF CERTAIN VETERINARY DRUG RESIDUES IN FOOD. Fifty-second report of the Joint FAO / WHO Technical Report Series No. 891. WHO, Geneva. Price: Sw. fr. 20; In developing countries Sw. fr. 14.

This report consists of the recommendations of the Fifty-second meeting of the Joint FAO / WHO Expert Committee on the safety of residues of certain veterinary drugs and on the maximum levels for such residues. It presents four parts : (1) Introduction, (2) General considerations, (3) Comments on residues of specific veterinary drugs and (4) Recommendations.

In the introductory section, the specific tasks before the Committee are mentioned and these are : (a) to elaborate further principles for evaluating the safety of residues of veterinary drugs in food and for establishing Acceptable Daily Intakes (ADIs) and MRLs for such residues, when the drugs under consideration are administered to food-producing animals in accordance with good practice in the use of veterinary drugs (b) to evaluate the safety of certain veterinary drugs and (c) to discuss matters of interest arising from the report of the Eleventh Session of the Codex Committee on Residues of Veterinary Drugs in Foods.

Section 2 focusses on general considerations with regard to principles governing the safety evaluation of residues of veterinary drugs in food, deletion of cyhalothrin, metrifonate, permethrin, temefos, tilmicosin and abamectin from the agenda, addition of neomycin and carazolol to the agenda for evaluation, evaluation of antimicrobial agents, evaluation of the effects of veterinary drug residues on the human intestinal micoflora, safety of residues at the injection site, MRLs for veterinary drug residues, MRLs for minor animal species, guidelines for identifying target tissues for residue analysis of food animal products in the international trade, requirements for validation of analytical methods and harmonization with the Joint FAO / WHO meeting on Pesticide Residues of substances used both as veterinary drugs and as pesticides.

Section 3 is mainly concerned on comments related to the residues of specific veterinary drugs. The Committee considered for the first time two insecticides (Deltamethrin and Phoxim) and one production aid (Porcine Somatotropins). It reconsidered one β-adrenoceptor - blocking agent (Carazolol), one anthelminthic agent (Doramectin), four antimicrobial agents (Dihydrostreptomycin and Streptomycin, Neomycin, Thiamphenicol), three production aids (Estradiol-17 β, progesterone and testosterone) and one tranquilizing agent (Azaperone).

Section 4 provides the recommendations on the agenda discussed during the meeting. These are briefly presented below.

(1) *Carazolol :* ADI 0 - 0.1 µg/kg of body weight, Acute RfD – 0 - 0.1 µg/kg of body weight. It may be noted that unless appropriate measures are taken to ensure that the concentrations of residues at the injection site do not result in intake exceeding the acute RfD, use of Carazolol during the transport of animals to slaughter will not be consistent with safe use of the drug.

(2) *Doramectin :* ADI 0.05 µg/kg of body weight. Recommended MRLs (µg/kg) are (a) Cattle : muscle 10, liver 100, kidney 30 and fat 100. The Committee noted the high concentration of residues at the injection site.

(3) *Dihydrostreptomycin and streptomycin :* ADI 0 - 50 µg/kg (combined residue of dihydrostreptomycin and streptomycin). The Committee suggested temporary MRL (both the compounds) in milk as 200 µg/kg.

(4) *Neomycin :* ADI 0 - 60 µg/kg of body weight. The MRL for kidney is increased from 5000 µg/kg to 10000 µg/kg to permit practical withdrawal times for all target animal species. Temporary designation for the MRLs in pigs, sheep, goats, turkeys, ducks and chicken is removed.

(5) *Thiamphenicol :* ADI 0 - 5 µg/kg of body weight. Temporary MRLs for the edible tissues of cattle and chicken were withdrawn. Temporary MRLs (µg/kg) were given for pig (muscle 50, liver 100, kidney 500 and fat 50) and fish (muscle with naturally adhering skin 50).

(6) *Deltamethrin :* ADI 0 - 10 µg/kg. The Committee maintained the same MRLs recommended by the 1990 Joint FAO / WHO Meeting on Pesticide Residues. The Committee felt that the concentrations of residues in muscle, milk and eggs were less than twice the limit of quantification of the analytical methods used and therefore recommended MRLs based on the sensitivity of the methods. These "guidance MRLs" are 30 µg/kg for muscle of cattle, sheep, chicken and salmon and for cow's milk and chicken's egg, expressed as parent drug.

(7) *Phoxim :* ADI 0 - 4 µg/kg of body weight. Temporary MRLs are indicated.

(8) *Estradiol-17 β, progesterone and testosterone :* ADI 0 - 0.05 µg/kg of body weight for estradiol-17 β; 0 - 30 µg/kg of body weight for progesterone and 0 - 2 µg/kg of body weight for testosterone. MRLs : "Not Specified" for edible tissues of cattle (applied to estradiol-17 β, progesterone and testosterones). It may be noted that the presence of drug residues in the named animal product does not present a health concern and that there is no need to specify a numerical MRL.

(9) *Porcine somatotropins :* ADI "Not Specified" (applied to Grolene, Reporcin and Somagrepor); MRLs "Not Specified" for edible tissues in pigs (applied to Grolene, Reporcin and Somagrepor).

(10) *Azaperone :* The new information provided on a method of analysis for azaperone is insufficient with regard to specificity, accuracy and reproducibility. The Committee forwarded the information to the Codex Committee on Residues of Veterinary Drugs in Foods for consideration.

The report is a very useful compendium for quality control laboratories, R & D organizations and academic institutions.

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